WEST Search History

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Hide?	Set Name	Query	Hit Count
		DB=PGPB; PLUR=YES; OP=OR	
☐	L1	20020192239	1
☐	L2	l1 and (sore$ or ulcer$)	1
☐	L3	l1 and decub$	0
☐	L4	decub$ or (pressure near2 (sore$ or ulcer$))	2376
☐	L5	bottox or bot-tox or botox or bo-tox or botn or btxa or btx-a or bont$2 or botulin or botulinum or botulism or bontinolysin or bontin-olysin or neurotoxin	4729
☐	L6	L5 and l4	594
☐	L7	L5 same l4	2
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☐	L8	bottox or bot-tox or botox or bo-tox or botn or btxa or btx-a or bont$2 or botulin or botulinum or botulism or bontinolysin or bontin-olysin or neurotoxin	14802
☐	L9	decub$ or (pressure near2 (sore$ or ulcer$))	7296
☐	L10	L9.clm. and l8.clm. not l7	0
☐	L11	L9 and l8 not l7	750
☐	L12	(L9 same l8) not l7	3
☐	L13	(L9 same l8)	5
☐	L14	L9.ti,ab,clm. and l8.ti,ab,clm. not l13	0
☐	L15	l8 near50 (spasticity or spasm or cramping or cramp or paralyze or paralysis or paralyzing)	587
☐	L16	L15 same dos$	217
☐	L17	minimal or partial or reducing or reduction or minimally or without or with-out	9137361
☐	L18	L17 same l16	59
☐	L19	l18 and l9	0
☐	L20	l18 and ulcer$	21
☐	L21	l18 and wound$	27
☐	L22	l21 not l20	15
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☐	L28	24419	2
☐	L29	wo and 2000 and botulinum	89
☐	L30	200024419	2

END OF SEARCH HISTORY

Items 1 - 3 of 3 One page.

1: Brin MF. Related Articles, Links

Dosing, administration, and a treatment algorithm for use of botulinum toxin A for adult-onset spasticity. Spasticity Study Group.
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3: Jankovic J, Brin MF. Related Articles, Links

Botulinum toxin: historical perspective and potential new indications.
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PMID: 9826986 [PubMed - indexed for MEDLINE]

L20: Entry 7 of 21 File: PGPB Oct 28, 2004

DOCUMENT-IDENTIFIER: US 20040213815 A1
TITLE: Clostridial toxin treatment for dermatillomania

Summary of Invention Paragraph:
[0055] Additionally, the finger biting, lip biting and tongue biting self mutilation behaviors of Lesch Nyhan syndrome have been treated by injecting a botulinum toxin into the chewing or clenching muscles of the mouth in one patient. Dabrowski E., et al, Botulinum toxin as a novel treatment for self-mutilation in Lesch-Nyhan syndrome, Ann Neurol 2002 September; 52 (3 Supp 1): S157. Injection of the fingers, lips or tongue is believed contraindicated because of the ulceration and sensitivity of these extremities due to the injurious behaviors of the syndrome.

Summary of Invention Paragraph:
[0056] Furthermore, a botulinum toxin has been used to treat focal dystonic tics or muscle spasms of Tourette's syndrome. Jankovic, J., Botulinum toxin in the treatment of tics associated with Tourette's syndrome, Neurology 1993 April; 43 (4 Supp 2): A310; Jankovic, J., Botulinum toxin in the treatment of dystonic tics, Mov Disord 1994 May; 9(3): 347-9, and; Krauss J., et al., Severe motor tics causing cervical myelopathy in Tourette's syndrome, Mov Disord 1996; 11(5): 563-6. These publications indicate that a botulinum toxin can act to treat a Tourette's syndrome tic both by reducing the force of contraction necessary to generate the muscle movement (i.e. by a partial paralysis of the tic involved muscles) as well as by an inhibition or resolution of the premonitory symptoms (i.e. by removing the urge to carry out or to accomplish the tic) which precede the tic. Unfortunately, significant neck pain, neck weakness and neck pain was reported in some of the Tourette's syndrome patient's administered a botulinum toxin to treat a neck tic. Additionally, the literature is contradictory with regard to use of a botulinum toxin to treat a Tourette's syndrome tic, as others have reported no relief upon use of botulinum toxin to treat a Tourette syndrome tic, even at dose levels that caused muscle weakness or paralysis. Chappell, P. B., et al., Future therapies of Tourette syndrome, Neurol Clin 1997 May; 15(2): 429-50, at 444.

Detail Description Paragraph:
[0123] An eight year old boy with mild mental retardation bites his fingers hands regularly and his fingers have become ulcerated. He mother reports that he will bite his fingers continuously unless restrained. The patient is treated by intramuscular injection of 3 units of a botulinum toxin type A (i.e. BOTOX.RTM.) into the base of each finger on each hand. Alternately, the forearm muscles can be injected bilaterally with 10 units of the botulinum toxin. Within 1-7 days after toxin administration the finger biting has completely subsided and resolved. His fingers heal and this alleviation of his condition persists for 4-6 months. For extended therapeutic relief (1 to 5 years), one or more polymeric implants incorporating a suitable quantity of a botulinum toxin type A can be placed at the target tissue site.

L20: Entry 16 of 21 File: PGPB Jun 12, 2003

DOCUMENT-IDENTIFIER: US 20030108597 A1
TITLE: Application of lipid vehicles and use for drug delivery

Summary of Invention Paragraph:
[0004] Interstitial cystitis (IC) is characterized by bladder pain, irritative voiding symptoms, and sterile urine (see R. Doggweiler-Wiygul et al., 2000, Curr. Rev. Pain 4(2):137-41). In IC, the bladder wall shows inflammatory infiltration with mucosal ulceration and scarring that causes smooth muscle contraction, diminished urinary capacity, hematuria, and frequent, painful urination. Although the pathogenesis of IC is uncertain, it seems likely that a dysfunctional epithelium results in the transepithelial migration of solutes, such as potassium, which depolarizes sensory nerves, and produces the symptoms (C. L. Parsons et al., 1991, J. Urol. 145:732; C. L. Parsons et al., 1994, J. Urol. 73:504; G. Hohlbrugger, 1999, Br. J. Urol. 83(suppl. 2):22; C. L. Parsons et al., 1998, J. Urol. 159:1862). Previous reports have shown that IC patients have defects in the glycosaminoglycan (GAG) layers of the uroepithelium (C. L. Parsons et al., 1991, J. Urol. 145:732; C. L. Parsons et al., 1994, J. Urol. 73:504; G. Hohlbrugger, 1999, Br. J. Urol. 83(suppl. 2):22). Thus, therapies that restore the mucosal lining or surface GAG layer, e.g., administration of heparine, hyaluronic acid, or pentosanpolysulfate, can reduce the leakage of irritant and result in palliation of IC symptoms (see, e.g., C. L. Parsons et al., 1994, Br. J. Urol. 73:504; J. I. Bade et al., 1997, Br. J. Urol. 79:168; J. C. Nickel et al., 1998, J. Urol. 160:612).

Summary of Invention Paragraph:
[0014] There are seven known serotypes of botulinum toxins (designated A-G). The serotypes differ in their cellular targets, potency, and duration of action, but all exert their paralytic effect by inhibiting acetylcholine release at the neuromuscular junction (see M. F. Brin, 1997, Muscle Nerve 20(suppl 6):S146-S168). Each serotype acts by cleaving one or more proteins involved in vesicle transport and membrane fusion. For example, botulinum toxin A is internalized by endocytosis at the axon terminal, where it is fully activated by disulfide reduction reactions, and it targets SNAP-25 (see M. F. Brin, 1997, Muscle Nerve 20(suppl 6):S146-S168). The extent of botulinum toxin-mediated paralysis depends on the dose, volume, and serotype employed. Botulinum toxin A causes reversible denervation atrophy that is typically terminated by axon sprouting within 2-6 months (see M. F. Brin, 1997, Muscle Nerve 20(suppl 6):S146-S168).

WO 00/24419
Wound healing

See search notes

toxin to use in the practice of the present invention is botulinum toxin type A.

def.

A method according to my invention can be carried out by administration of a Clostridial toxin to a patient with, or who is predisposed to developing, a pressure sore. The Clostridial toxin used is preferably a botulinum toxin (as either a complex or as a pure [i.e. about 150 kDa molecule], such as a botulinum neurotoxin A, B, C1, D, E, F or G. Administration of the Clostridial toxin can be by a transdermal route (i.e. by application of a Clostridial toxin in a cream, patch or lotion vehicle), subdermal route (i.e. subcutaneous or intramuscular) or intradermal route of administration.

Except when treating a pressure sore related to contractures or spasticity, the dose of a Clostridial toxin used according to the present invention is less than the amount of toxin that would be used to paralyze a muscle, since the intent of a method according to the present invention is not to paralyze a muscle but to treat a pressure sore.

The following definitions apply herein:

"About" means approximately or nearly and in the context of a numerical value or range set forth herein means ±10% of the numerical value or range recited or claimed.

"Alleviating" means a reduction in the occurrence of a pressure sore symptom. Thus, alleviating includes some reduction, significant reduction, near total reduction, and total reduction of a pressure sore symptom. An alleviating effect may not appear clinically for between 1 to 7 days after administration of a Clostridial neurotoxin to a patient.

DOCUMENT-IDENTIFIER: US 20050238667 A1
TITLE: Botulinum toxin pharmaceutical compositions with enhanced potency with regard to a reference botulinum toxin pharmaceutical composition

Summary of Invention Paragraph:
[0077] Animals may also cause severe injury to themselves due to what would be a mild condition in humans. Wounds are often exacerbated by stall rubbing in horses resulting in insufficient healing and chronic sores. Sores and skin irritation can result in serious infection in this manner. Other maladies not seen in human patients exist in animals. For example, flies and parasites cause discomfort and act as vectors for disease. Dogs often require hospitalization and or restraint due to biting and scratching related to fleas and mange. Collars are often placed around the animal's neck to prevent the animal from scratching ears affected by mites or surgical procedures. These maladies, if mediated for a time sufficient for the affected area to heal or the parasite to be removed, can be avoided, relieving the animal of suffering and symptoms.

Detail Description Paragraph:
[0218] Compositions containing other serotypes of botulinum toxin may contain different dosages of the botulinum toxin. For example, botulinum toxin type B may be provided in a composition at a greater dose than a composition containing botulinum toxin type A. In one embodiment of the invention, botulinum toxin type B may be administered in an amount between about 1 U/kg and 150 U/kg. Botulinum toxin type B may also be administered in amounts of up to 20,000 U (mouse units, as described above). In another embodiment of the invention, botulinum toxin types E or F may be administered at concentrations between about 0.1 U/kg and 150 U/kg. In addition, in compositions containing more than one type of botulinum toxin, each type of botulinum toxin can be provided in a relatively smaller dose than the dose typically used for a single botulinum toxin serotype. The combination of botulinum toxin serotypes may then provide a suitable degree and duration of paralysis without an increase in diffusion of the neurotoxins (e.g. see U.S. Pat. No. 6,087,327).

Previous Doc Next Doc Go to Doc#

to modify involuntary natural behaviors, for example ear and tail twitching, in show horses for example. Such drastic procedures are performed for cosmetic reasons. No other humane remedy currently exists to correct such undesirable traits. These procedures permanently damage the animals.

[0077] Animals may also cause severe injury to themselves due to what would be a mild condition in humans. Wounds are often exacerbated by stall rubbing in horses resulting in insufficient healing and chronic sores. Sores and skin irritation can result in serious infection in this manner. Other maladies not seen in human patients exist in animals. For example, flies and parasites cause discomfort and act as vectors for disease. Dogs often require hospitalization and or restraint due to biting and scratching related to fleas and mange. Collars are often placed around the animal's neck to prevent the animal from scratching ears affected by mites or surgical procedures. These maladies, if mediated for a time sufficient for the affected area to heal or the parasite to be removed, can be avoided, relieving the animal of suffering and symptoms.

[0078] A significant barrier in treating animals and promoting their recovery from injury or surgery is due to an inability to instruct the animal about proper rest and rehabilitation procedures. The difficulty of immobilizing an animal may result in re-injury, self-injury, and/or improper healing. Minor injuries often become catastrophic due to the difficulty of immobilizing animals during and after treatment. Many times the animal must be destroyed. In addition, wild animals (e.g., non-domestic animals) that pose a danger to others or themselves may need to be immobilized to safely return the animal to its native environment. Typically, these animals are tranquilized and moved, or are killed.

[0079] Another problem when treating animals arises from the difficulty in administering proper dosages. Animals do not readily swallow oral dosage forms of medicine and often offer violent resistance to injections. Frequent application of short-acting drugs may therefore not be possible. Accordingly, as opposed to humans, animals may require long-acting, single injection pharmaceuticals to receive adequate benefit from the drug.

[0080] While pharmaceutical compositions containing human protein may not present problems from a single administration, except in certain individuals or species, repeated dosing may initiate immune responses in animals, including anaphylactic shock. As discussed above, current formulations of *botulinum* toxin include human serum albumin (HSA) as a stabilizing excipient. These formulations may present serious problems for veterinary use because the presence of a human protein may induce immunogenic responses in other animal species, limiting the efficacy, utility, and safety of the drug. Formulations which do not contain HSA as an excipient, however, should not produce these antibodies. The lack of suitable formulations of *botulinum* toxin for use in treating non-human animals has impeded the development of methods of using *botulinum* toxin in veterinary medicine.

[0081] Indeed, the majority, if not all, of research regarding the therapeutic aspects of *botulinum* toxin has focused on the use of *botulinum* toxin for treating human diseases or ailments. As a necessary precursor, *botulinum* toxin has experimentally been administered to animals to examine its safety and efficacy in animal models of human diseases. For example, *botulinum* toxin type B has been administered to mice to treat thermal hyperalgesia (Tsuda et al., (1999) "In vivo pathway of thermal hyperalgesia by intrathecal administration of β-methylene ATP in mouse spinal cord: Involvement of the glutamate-NMDA receptor system", Br. J. Pharmacol., 127(2):449-456). In addition, *botulinum* toxin type A has been:

[0082] administered to piglets to relieve lower esophageal sphincter (LES) pressure (U.S. Pat. No. 5,437,291, entitled "Method for treating gastrointestinal muscle disorders and other smooth muscle dysfunction");

[0083] injected into gastric wall of rats to treat obesity (Gui et al., (2000) "*Botulinum* toxin injected in the gastric wall reduces body weight and food intake in rats", Aliment Pharmacol Ther., 14(6):829-834);

[0084] used to assess the efficacy of the neurotoxin to produce better scar formation from facial cosmetic surgery in non-human primates (Gassner et al., (2000) Plast. Reconst. Surg., 105(6):1948-1953);

[0085] administered to rats to examine its ability to reduce pain (Aoki et al., (2000) "Methods for treating pain", U.S. Pat. No. 6,113,915;

[0086] administered to rats and dogs to alter vocal cord dynamics for treating spasmodic dysphonia and vocal cord paralysis in humans (Inagi et al., (1998) "Physiologic assessment of *botulinum* toxin effects in the rat larynx", Laryngoscope, 108(7):1048-1054; and Cohen and Thompson (1987) "Use of *botulinum* toxin to lateralize true vocal cords: a biochemical method to relieve bilateral abductor vocal cord paralysis", Ann. 0 to 1. Rhinol. Laryngol., 96(5):534-41); and

[0087] administered to the obicularis oculi muscle of the guinea pig to examine the efficacy of the toxin to treat eye lid spasms (Horn et al., (1993) "*Botulinum* toxin paralysis of the orbicularis oculi muscle. Types and time course of alterations in muscle structure, physiology and lid kinematics", Exp. Brain Res., 96(1):39-53).

[0088] In addition, a *botulinum* toxin has been administered to rabbits to assess the immunogenicity of the toxin when conjugated with human serum albumin (HSA).

[0089] Because the administration of *botulinum* toxin to animals has only been examined experimentally as a model for treating humans with *botulinum* toxin, researchers have not been concerned with long-term effects of the neurotoxin on animals. In particular, the art has not addressed the immunogenicity of the *botulinum* toxin compositions in the non-human animals receiving the neurotoxin. Thus, it would be beneficial to have a neurotoxin composition with reduced immunogenicity for use in veterinary care, and convenient and effective methods for immobilizing or treating non-human animals.

[0090] What is needed therefore is a *botulinum* toxin containing pharmaceutical composition which is free of animal derived proteins such as a blood pooled or blood fraction derived serum albumin or gelatin.

DRAWINGS

[0091] FIG. 1 is a graph (a chromatogram) which shows the molecular weight distribution (higher molecular weight

US006447787B1

(12) **United States Patent**
Gassner et al.

(10) **Patent No.: US 6,447,787 B1**
(45) **Date of Patent: Sep. 10, 2002**

(54) **METHODS FOR ENHANCING WOUND HEALING**

(75) Inventors: **Holger G. Gassner**, Erlangen (DE); **David A. Sherris**, Rochester, MN (US)

(73) Assignee: **Mayo Foundation for Medical Education and Research**, Rochester, MN (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/807,793**

(22) PCT Filed: **Oct. 15, 1999**

(86) PCT No.: **PCT/US99/24182**

§ 371 (c)(1),
(2), (4) Date: **Apr. 18, 2001**

(87) PCT Pub. No.: **WO00/24419**

PCT Pub. Date: **May 4, 2000**

Related U.S. Application Data

(60) Provisional application No. 60/105,688, filed on Oct. 27, 1998.

(51) **Int. Cl.**[7] **A61K 39/08**; A61K 39/02

(52) **U.S. Cl.** **424/247.1**; 424/239.1; 424/236.1

(58) **Field of Search** 424/247.1, 239.1, 424/236.1

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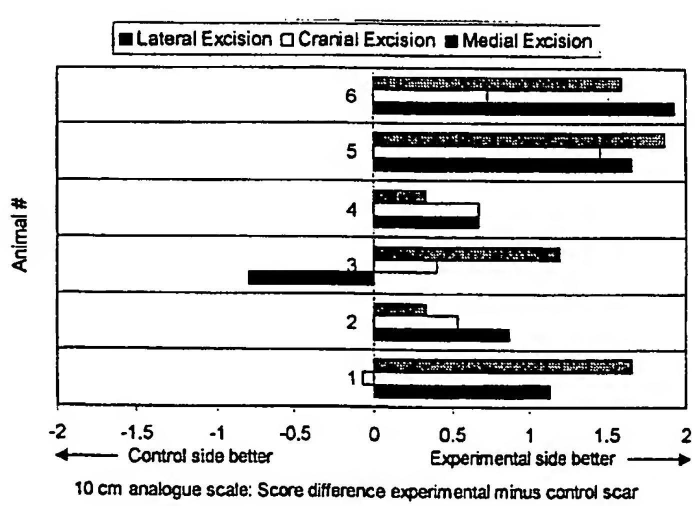
Primary Examiner—Frederick Krass
Assistant Examiner—Donna Jagoe
(74) *Attorney, Agent, or Firm*—Fish & Richardson, P.C., P.A.

(57) **ABSTRACT**

A method for treating a patient having a wound is described. The method includes administering an amount of a chemodenervating agent such that healing of the wound is enhanced. The method is illustrated by detailing the mean differences of the scores of the paired experimental and control scars across three observers.

34 Claims, 1 Drawing Sheet



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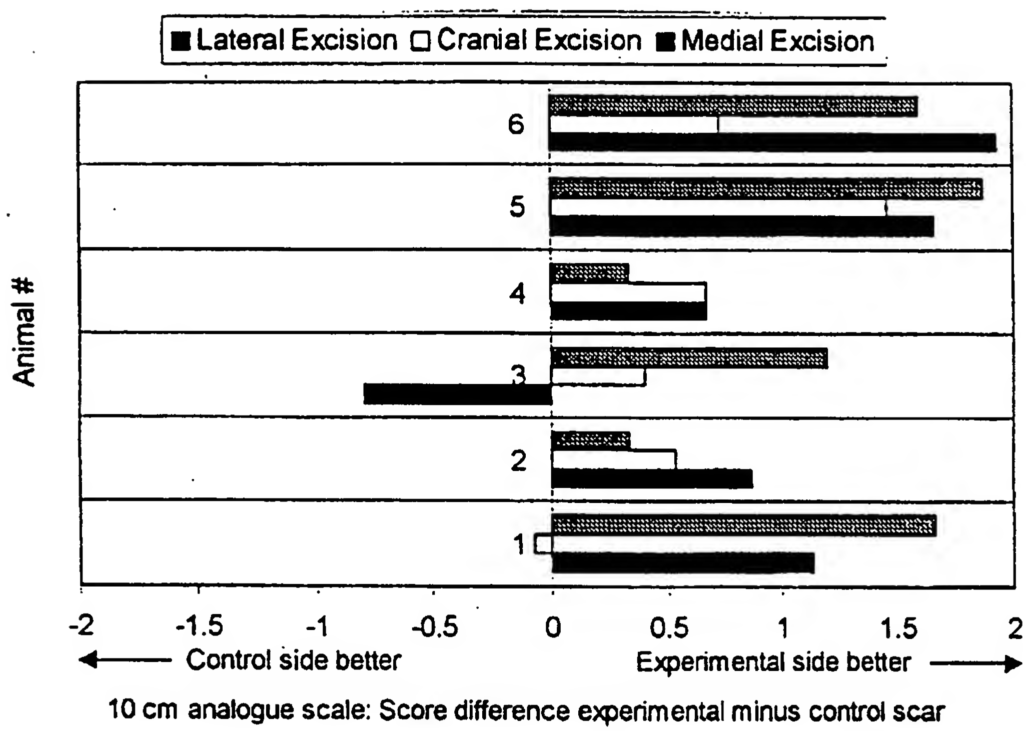
Lateral Excision
Cranial Excision
Medial Excision
Animal #
6
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Control side better
Experimental side better
10 cm analogue scale: Score difference experimental minus control scar

METHODS FOR ENHANCING WOUND HEALING

This application is the national stage (371) of PCT/US99/24182 filed Oct. 15, 1999 which claims the benefit of provisional application No. 60/105,688 filed Oct. 27, 1998.

TECHNICAL FIELD

The invention relates to a method for enhancing wound healing.

BACKGROUND OF THE INVENTION

Immobilization is a basic therapeutic principle in wound healing, common to the treatment of lesions of all kinds. Casts, plates, and sutures minimize the negative effects of muscle tension on healing tissues. Since tension is one of the chief factors determining the degree of scar formation, this principle also holds true in skin lesions. The carefully-planned execution of an elective skin incision frequently achieves the best aesthetic result.

Surgeons have been seeking techniques and methods to reduce excessive scar formation, especially in the face. Many approaches have been undertaken to overcome the negative influence of muscular tension on the wound healing process, including various suture techniques, steroid injections, undermining wound edges, and placing incisions in a line parallel to relaxed skin tension lines (RSTLs).

The etiology of skin tension lines, first described more than a century ago, has been subject to controversy over the years. There is general agreement, however, that skin tension lines influence the healing of incisions according to their relative positions. There is evidence that the formation of RSTLs is a dynamic process over time. Studies on fetal calves and human fetal skin suggest that RSTLs are not genetically determined, but represent a change of texture of the skin secondary to extrinsic and/or intrinsic forces. Lorenz, H. P. et al., *Development*, 114(1):253–259, (1992). This change in texture gives skin certain mechanical characteristics that are retained even when excised. Muscle tension is thought to be a major factor in the formation of RSTLs.

Increased skin tension has a negative effect on wound healing, causing hypertrophic scars or wound dehiscence. See, for example, Sherris, D. A. et al., *Otolaryngologic Clinics of North America*, 28(5): 1957–1968, 1995. Repeated microtrauma, caused by continuous displacement of injured tissue, induces a prolonged inflammatory response and an increased metabolic activity during the healing process. As a consequence, extracellular deposition of collagen and glycosaminoglycans can intensify and lead to hypertrophic scars. The incidence of hypertrophic scars is higher in certain anatomic areas where there is increased muscular movement. McCarthy, J. G., Plastic Surgery, 1990, Vol. 1, Philadelphia, W B Saunders, page 44.

SUMMARY OF THE INVENTION

The invention is based, in part, on a new therapy for management of both traumatic and iatrogenic wounds, which includes the elimination of the tension acting on the wound. The new therapy includes injection of a chemodenervating agent to paralyze muscles capable of exerting tension on such wounds, providing better wound healing with minimal scar development. In addition, early immobilization in elective procedures also allows a surgeon to use finer sutures, further improving the cosmetic result.

In one aspect, the invention features a method for treating a patient having a wound (e.g., a facial wound). The method includes locally administering an amount of a chemodenervating agent such that healing of the wound is enhanced. The chemodenervating agent can be, for example, a botulinum toxin, saxitoxin, tetanus toxin, or tetrodotoxin, and is typically administered by injection. The botulinum toxin can be botulinum toxin A, B, C, D, E, F, or G, and in particular botulinum toxin A or B. The method further can include administering an amount of a local anesthetic agent and/or a local vasoconstrictive agent effective to enhance wound healing. Local anesthetic agents such as lidocaine, bupivacaine, or mepivacaine, or local vasoconstrictive agents can be administered prior to injection with the chemodenervating agent or simultaneously with the chemodenervating agent.

A composition having a chemodenervating agent, a local anesthetic, and a local vasoconstrictive agent also is featured.

In another aspect, the invention features an article of manufacture that includes packaging material and an amount of a chemodenervating agent. The packaging material includes a label that indicates the chemodenervating agent is useful for treating a patient having a wound. Administration of the chemodenervating agent enhances healing of the wound. The chemodenervating agent can be a botulinum toxin such as botulinum toxin A. The article of manufacture also can include a local anesthetic agent or a vasconstrictive agent.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used to practice the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graph that indicates the mean differences of the scores of the paired experimental and control scars across three observers.

DETAILED DESCRIPTION

As described herein, the cosmetic appearance of a scar is influenced by underlying muscle activity during the wound healing process. Paralysis of the underlying muscle activity increases the rate of healing and yields a better cosmetic result. Without being bound by a particular mechanism, locally induced paralysis of the musculature subjacent to a cutaneous defect is thought to minimize the repetitive tensile forces on the wound edges, resulting in superior cosmetic outcome in the resultant scar.

Thus, the invention provides a method for treating a patient having a wound that includes locally administering an amount of a chemodenervating agent effective to enhance wound healing in the patient. As used herein, "chemodenervating agent" refers to any agent that interrupts nerve

impulse transmission across the neuromuscular junction, blocks the release of neurotransmitters, or alters the action potential at the voltage gated sodium channel of neurons, sufficient to reduce tension within muscles in and near a wound site.

As used herein, "wound" refers to skin, tendon, or bone wounds, and can include inflammatory lesions or other lesions adversely affected by muscle tension or movement. Skin wounds include, for example, facial lacerations such as those introduced by trauma (i.e., a car accident), or iatrogenic, such as surgically introduced incisions. In particular, surgically introduced incisions include scar revision excision surgery. As such, a skin wound includes elective incisions and nonelective incisions. Skin wounds may be relatively favorable or unfavorable. As used herein, "favorable wound" refers to an incision or laceration that is relatively parallel to RSTLs, whereas "unfavorable wound" refers to an incision relatively perpendicular to RSTLs. Both favorable and unfavorable wounds benefit from the methods described herein. Tendon wounds include, for example, ruptured or injured tendons and tendinitis.

Bone wounds include favorable and unfavorable fractures. A "favorable fracture" refers to a fracture that is not prone to displacement of one or more fragments of the fracture by muscle pull, whereas an "unfavorable fracture" refers to a fracture that is prone to displacement of one or more fragments by muscle pull. The treatment for a fracture can be facilitated if muscle tension on the affected fracture is minimized. Thus, the treatment becomes less invasive, less time consuming and/or less costly. For example, with a fractured elbow, the triceps muscle can displace the bone fragments. An alternative to surgical repair includes use of percutaneous wires to hold the bones in place, and relaxation of the triceps muscle by paralysis with a chemodenervating agent. Use of wires and a chemodenervating agent may reduce or avoid surgery and/or the accompanying general anesthesia.

The methods described herein enhance wound healing by minimizing the adverse effect of muscle tension and movement on the wound, as well as improving cosmetic appearance through reduced scar development. In addition, inflammation may be reduced during the healing process.

Chemodenervating Agents

Non-limiting examples of chemodenervating agents include botulinum toxin, saxitoxin, tetanus toxin, and tetrodotoxin. Suitable botulinum toxins include, for example, botulinum toxins A, B, C (C1 and C2), D, E, F, or G. Botulinum toxins A, B, and F are particularly useful. Botulinum toxin A is a potent drug that produces temporary muscular paralysis when injected locally. Botulinum toxin A has been used in the treatment of a wide range of disorders associated with involuntary muscle contraction. It has been demonstrated to be effective in treating focal dystonias such as blepharospasm, nondystonic disorders such as hemifacial spasms, disorders of conjugate eye movement such as strabismus and nystagmus, spasticity disorders such as multiple sclerosis and cerebral palsy, and for disorders of localized muscle spasm. In addition, botulinum toxin A has been used to treat age related rhytids of the upper face. Botulinum toxin A is safe and effective to use, and is relatively painless with rare side effects characterized as mild and transient. Onset of action takes place within 24 to 72 hours after injection and lasts 2 to 6 months. Botulinum toxin A is available commercially, e.g. from Allergan, Inc. (Irvine, Calif. Botox®) and Speywood Pharmaceuticals (England, Dysport®).

Dosages of botulinum toxin A required for local immobilization typically do not exceed 1 unit toxin per kg body weight and are safe. Primate studies have indicated that no systemic effects are observed at dosages below 33 units/kg body weight. See, for example, Scott and Suzuki, *Mov. Disord.*, 1988, 3:333–335.

Botulinum toxins B and F also have been used for dystonia patients. Greene, P. E. et al., *Mov. Disord.*, 1996, 11(2):181–184; and Truong, D. D. et al., *Mov. Disord.*, 1997, 12(5):772–775. Botulinum toxin B is available from Elan Corporation (Dublin, Ireland, Neurobloc®).

Botulinum toxins also can be obtained by purifying the toxins from strains of *Clostridium botulinum*, using standard techniques. For example, botulinum toxin A can be produced in a Hall strain using a nutritive medium containing casein digest, yeast extract, and dextrose. After lysis of the culture, the toxin is released into the medium and activated by proteases, and then is acid precipitated. Further purification can include extraction with a sodium phosphate buffer, ethanol precipitation, and crystallization in ammonium sulfate. See, for example, Schantz, E. J. and Johnson, E. A., *Microbiol. Rev.*, 1992, 56(1):80–99.

Other chemodenervating agents such as saxitoxin, tetanus toxin, and tetrodotoxin are also suitable. The paralysis induced by saxitoxin, however, does not last as long as that induced by botulinum toxin. Consequently, repeated injections of saxitoxin may be needed. Saxitoxin can be purified by known procedures. See, for example, Schantz, E. J. et al., *J. Am. Chem. Soc.*, 1975, 97:1238–1239. Tetanus toxin can decrease acetylcholine release in cholinergic peripheral nerves when injected locally. Dreyer, F., Peripheral actions of tetanus toxin, p. 179–202, In: Botulinum neurotoxin and tetanus toxin. Academic Press, Inc., San Diego. L. L. Simpson (ed.). Tetanus toxin also can enter the central nervous system where it causes uncontrolled muscle spasms. When tetanus toxin is employed in the methods described herein, precautions must be taken to ensure local response. Matsuda, M. et al., *Biochem. Biophys Res. Commun.*, 1982, 104:799–805; and Habermann, E. et al., *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 1980, 311:33–40. Tetanus toxin can be purified by standard procedures. See, for example, Robinson, J. P., *Methods Enzymol.*, 1988, 165:85–90. Tetrodotoxin blocks the sodium channel of excitable membranes of nerve and muscle tissues, and can be purified using routine techniques. See, for example, Yotsu, M. et al., *Toxicon*, 1987, 25:225–228.

Local administration of the chemodenervating agents typically occurs by subcutaneous (SQ), intramuscular (IM), perimuscular injection, or percutaneous instillation (e.g., air gun or skin patch). When chemodenervating agents are injected SQ, the agent reaches the muscle by perfusion. For elective incisions, the chemodenervating agent can be administered prior to making an incision, while making an incision, or after an incision has been made.

Administration of Local Anesthetics and Local Vasoconstrictive Agents

The method of treatment further can include administering either a local anesthetic agent or a local vasoconstrictive agent, or both. Such agents can be administered prior to injection of the chemodenervating agent or simultaneously with the injection of the chemodenervating agent. Local anesthetics block nerve conduction, and can cause sensory and motor paralysis in localized area. Local anesthetics have a rapid onset of action, and therefore reduce muscle tension on the wound almost immediately as well as reduce pain associated with the injection. The extent of muscular paralysis achieved by a local anesthetic agent is helpful in predicting the extent of paralysis that can be achieved by subsequent injection of a chemodenervating agent into the

same injection site. Thus, possible local side effects, such as diffusion of the chemodenervating agent to adjacent muscle groups, is prevented. Non-limiting examples of local anesthetic agents include lidocaine, bupivacaine, chloroporcaine etidocaine, or mepivacaine, and are available commercially. In addition, other amide types of local anesthetics can be used in the method. Suitable amounts of local anesthetics can be readily determined by a physician. For example, about 1 to 5 mls of lidocaine at a concentration of about 0.5%–about 2% can be injected. Administration of local anesthetics is particularly useful when incisions are introduced surgically, such as during scar reversion excision surgery.

Administration of a local vasoconstrictive agent results in a decreased hemoperfusion of the injected tissue. Thus, administration of a local vasoconstrictive agent can help prevent or control diffusion of the chemodenervating agent and minimize possible side effects, such as brow ptosis or incomplete eye closure from injection into the frontalis and/or corrugator supercilii muscles. Non-limiting examples of local vasoconstrictive agents include epinephrine and phenylephrine, and are available commercially. A suitable amount of a local vasoconstrictive agent can be readily determined by a physician. For example, 5 mls of epinephrine 1:100,000 or 1:200,000 typically is used for local vasoconstrictive action.

Compositions containing a chemodenervating agent and a local anesthetic, and/or a local vasoconstrictive agent, can be produced for applications in which it is desired to introduce chemodenervating agents and one or more other components simultaneously. Such compositions can be prepared, for example, by reconstituting a lyophilized component with a solution of another component. For example, lyophilized botulinum toxin can be reconstituted in a solution containing a local anesthetic and a local vasoconstrictive agent, or in a solution containing either a local anesthetic or a local vasoconstrictive agent. A composition containing lidocaine and epinephrine is commercially available, for example, from Astra. Typically, lidocaine is present at 0.5–2% and epinephrine is present at 1:100,000 to 1:200,000.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

EXAMPLE 1

Enhanced Wound Healing By Injection of a Chemodenervating Agent in Monkeys

In order to closely mimic the effects of muscle activity on human facial skin wounds, the use of an appropriate animal model was mandatory. Due to extensive skin laxity and inadequate mimetic musculature, established models like rats, pigs, and horses, were not ideal for this purpose. *Cynomolgus macaque* monkeys (*Macaca fascicularis*) were chosen as a model since the anatomy of their cranio facial and cutaneous anatomy resembles that of humans.

The study was approved by the Institutional Committee of Animal Care and Use at the Mayo Clinic and the animals were housed, cared for, and fed in compliance with the institutional guidelines. No animal was sacrificed. All procedures were performed with anesthesia consisting of Ketamine at 20 mg/kg IM (Ketaset®, Fort Dodge), Xylazine at 0.5 mg/kg IM (Rompun®, Bayer), and Isoflurane at 1% (Isoflurane®, Abbott).

The forehead was chosen for the excision site in the monkeys as the frontalis, procerus and corrugator supercilii muscles constantly exert tension on the forehead skin and paralysis of these muscles leads to no functional deficit. In order to minimize local variables, the experimental and control excisions were each planned in symmetric anatomic location in the same individual animal. Three Y-shaped excisions with their main axis perpendicular to the RSTLs were planned symmetrically in relation to the midline on each side of the forehead.

A template was used to determine the location and outline of the excisions to ensure maximal precision. An experienced facial plastic surgeon, blinded to the experimental conditions, performed all excisions. Using standard surgical technique, the skin and subcutaneous tissue was excised and the frontalis muscle was preserved in the base of the defects. Subsequently, one side of the forehead was randomly determined as experimental and the mimetic musculature adjacent to each excision on that side was injected under direct vision with 7 units of Botulinum Toxin A (Botox®, Allergan) in 0.9% saline (25 units/ml), resulting in a total dose of 21 units of Botulinum toxin A per half forehead. The control side was injected in the same fashion with an equal volume of 0.9% saline alone. All wounds were closed with a single 6-O Chromic Gut (Chromic Gut®, Ethicon) buried suture and multiple 5-O black monofilament Nylon (Ethilon®, Ethicon) superficial sutures. From the third day postoperatively, marked paralysis of the Botulinum toxin A treated side was observed in all six animals. Extraocular muscle movement and eyelid closure were not compromised.

Three experienced facial surgeons, who were not present during the surgical procedures, were used as blinded observers to evaluate the cosmetic appearance of the scars at 1, 4, and 12 weeks postoperatively. Care was taken to sedate the animals deeply for each assessment so the evaluators were not able to recognize the paralyzed side of the forehead.

First, the evaluators were asked to score each single scar on a 10 cm visual analogue scale. The 36 forehead scars (3 experimental scars and 3 control scars per animal) were evaluated by each assessor independently. In this scale, scars were rated from 1 to 10, with 0 being the worst and 10 being the best. At 1 and 4 weeks postoperatively, none of the blinded ratings revealed a significantly better cosmetic appearance of the experimental or the control wounds. The mean ratings of the three assessors at 12 weeks postoperatively reached a higher score on the experimental side in 16 of 18 of the symmetric pairs of scars (FIG. 1). The bars in FIG. 1 represent the mean differences of the scores of the paired experimental and control scars across the three observers. The mean score by assessor #1 was 9.4 for the experimental scars and 8.1 for the control scars; the mean score by assessor #2 was 8.0 for the experimental scars and 7.3 for the control scars; and the mean score by assessor #3 was 7.9 for the experimental scars and 7.3 for the control scars. The mean scores across the three assessors were 8.4 (SD 1.0) for the experimental side and 7.6 (SD 0.9) for the control side. The statistical assessment of an intervention effect was based on using the average rating across the three evaluators and fitting a two-factor (intervention, site) repeated measures analysis of variance model, taking into account the correlation of measurements obtained on the same animal. Based on this analysis, the scars on the experimental side were rated significantly better than the scars on the control side ($p<0.01$).

Secondly, the assessors were asked to examine the groups of 3 scars on either side of each animal's forehead (12 weeks postoperatively) and to rate each scar as better, equal to, or worse than its symmetric counterpart. A consensus score was derived from the majority of the votes. The experimen-

tal sides were assessed as better than the control sides in 6 of the 6 animals. Based on a two-tailed, one-sample binomial test, this result was statistically significant ($p<0.031$) (Table 1).

TABLE 1

Assessment of Scars

Animal	Assessor 1	Assessor 2	Assessor 3	Consensus Score
1	+	?	+	+
2	+	?	+	+
3	+	+	-	+
4	+	+	+	+
5	+	+	+	+
6	+	+	+	+

+ = Assessment of experimental side as better
− = Assessment of experimental side as worse
? = Assessment of both sides equal

Representative sections of the scars were excised 12 weeks postoperatively, using a 4 mm punch. The biopsy specimens were embedded in formalin, cut in 25 μm thick sections, and hematoxylin and Eosin stained for evaluation. Scars were classified as mature with no sign of inflammation or ongoing remodeling.

EXAMPLE 2

Enhanced Wound Healing by Botulinum Injection in Humans

A male patient (26 years of age, 82 kg) underwent scar revision excision surgery. The scar was located on the forehead approximately 2 cm lateral of the midline on the left, and approximately 3 cm cranial to the most superior extension of the orbital rim. Its direction was horizontal, giving it a favorable position relative to the wrinkle lines. The scar was a result of a trauma at age seven, and was closed at a tertiary referral center at the time.

The patient was placed in a supine position, and 5 ml of 0.5% lidocaine with 1:200,000 epinephrine was locally injected. The scar was excised and bleeding was controlled with monopolar cautery. Botulinum toxin A was injected (10 units) into the frontalis muscle under direct vision fanning out from the wound. The wound was closed using 6-0 Vicryl for deep and 6-0 Nylon for superficial sutures. An additional 7.5 units of botulinum toxin A were injected into the procerus and corrugator muscles bilaterally, as frowning caused distortion of the wound.

Approximately 24 hours after surgery, the patient developed marked paralysis of the injection muscles, and had lost the ability to wrinkle the forehead skin in an area of about 4 cm in diameter around the excision. The wound healed well in the early postoperative period. It was apparent that there was decreased movement and tension on the wound edges. The forehead wound of the patient healed without complications. Compared to the preoperative scar, the cosmetic appearance of the resulting scar 12 months postoperatively was excellent and superior to the initial scar.

EXAMPLE 3

Evaluation of Scars from Patients Injected with a Chemodenervating Agent Alone or in Combination with a Local Anesthetic

Healthy volunteers were informed about potential risks and side effects of the treatment. Formal written informed consent was obtained in accordance with the Mayo Institutional Review Board regulations. Prior to enrollment in the study, symmetry of frontalis, procerus, and corrugator supercilii function was assessed and subjects were only included in the study if there was no functional asymmetry present. The forehead of the subjects was divided by the midline into two symmetric sides, one serving as the control and the other as the experiment side. The side of the forehead which was to serve as control was determined randomly, and was injected with Botulinum Toxin A (Botox) reconstituted in 0.9% saline. The experimental side was injected with Botulinum Toxin A reconstituted in 1% or 2% lidocaine with 1:100,000 epinephrine. The combination of these agents with Botulinum toxin A was achieved by reconstituting 100 units of freeze dried Botulinum toxin A in 5 ml of 1% or 2% Lidocaine with 1:100,000 epinephrine solution (Xylocaine at 1% or 2% with epinephrine 1:100,000, Astra). This resulted in a dosage which is commonly utilized for each of these substances in routine clinical use (20 units Botulinum toxin per ml of 1% or 2% lidocaine with 1:100,000 epinephrine).

In order to assure symmetry and equality of the injections, the sites of injection were predetermined with a template. A predetermined amount and volume of toxin was injected into each location. After the injection, subjects were asked to evaluate the intensity of the pain resulting from the percutaneous injections for both sides of the forehead separately. This was done with a standardized questionnaire approximately 10 minutes after the injection. The pattern of muscular paralysis achieved by the local anesthetic plus Botox was compared to the pattern of paralysis resulting from Botox A alone at one week after the injection. The potency and duration of action of Botox A reconstituted in the vasoconstrictive and anesthetic agent was compared to Botox A reconstituted in 0.9% saline by serial observation until the return of facial muscular function. Subjects were photographed 5–15 minutes after injection, one week after injection, and monthly thereafter attempting maximal forehead muscle contracture.

Two particular examples of such injections are provided. A white female was injected with 20 units Botox in 1 ml 1% lidocaine with 1:100,000 epinephrine in the right side of the forehead and in exactly the same fashion with 20 units Botox, reconstituted in 0.9% saline in the left side of the forehead. A second white female was injected in the same manner, except that 2% lidocaine was used. Eight portions of 0.125 ml were injected into each side of the forehead and the sites of injection were determined by a template. Each subject immediately developed paralysis of the frontalis, procerus, and depressor supercilii muscles on the right side of the forehead. The pattern and extent of immediate muscular paralysis resulting from the immediate action of the local anesthetic drug (Lidocaine 1% or 2%) was predictable of the pattern and extent of delayed paralysis achieved by Botox one week later. The effect of the Botox-induced muscular paralysis faded in a symmetric fashion, indicating that the duration of Botox induced muscular paralysis was not affected by the addition of Lidocaine or epinephrine.

Other Embodiments

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

What is claimed is:

1. A method for treating a patient having an acute skin wound, said method comprising locally administering an amount of botulinum toxin in or in close proximity to said acute skin wound, such that healing of said skin wound is enhanced.

2. The method of claim 1, wherein said botulinum toxin is selected from the group consisting of botulinum toxin A, B, C, D, E, F, and G.

3. The method of claim 2, wherein said botulinum toxin is botulinum toxin A.

4. The method of claim 2, wherein said botulinum toxin is botulinum toxin B.

5. The method of claim 1, wherein said administering step is by injection.

6. The method of claim 5, wherein said botulinum toxin is subcutaneously injected.

7. The method of claim 5, wherein said botulinum toxin is intramuscularly injected.

8. The method of claim 5, wherein said botulinum toxin is percutaneously instilled.

9. The method of claim 1, said method further comprising administering a local anesthetic.

10. The method of claim 9, wherein said local anesthetic is lidocaine.

11. The method of claim 9, wherein said local anesthetic is bupivacaine.

12. The method of claim 9, wherein said local anesthetic is mepivacaine.

13. The method of claim 9, wherein said local anesthetic is administered prior to administration of said botilinum toxin.

14. The method of claim 9, wherein said botulinum toxin and local anesthetic are co-administered.

15. The method of claim 1, said method further comprising administering a local vasoconstrictive agent.

16. The method of claim 15, wherein said local vasoconstrictive agent is epinephrine.

17. The method of claim 1, said method further comprising administering a local anesthetic and a vasoconstrictive agent.

18. The method of claim 17, wherein said local anesthetic and said vasoconstrictive agent are administered prior to said botulinum toxin.

19. The method of claim 1, wherein said acute skin wound is a facial wound.

20. The method of claim 1, wherein said acute skin wound is a surgically introduced incision.

21. The method of claim 20, wherein said botulinum toxin is administered prior to making said surgically introduced incision.

22. The method of claim 20, wherein said botulinum toxin is administered while making said surgically introduced incision.

23. The method of claim 20, wherein said botulinum toxin is administered after said surgically introduced incision has been made.

24. The method of claim 1, wherein said acute skin wound is traumatically introduced.

25. The method of claim 1, wherein said acute skin wound is a favorable wound.

26. The method of claim 1, wherein said acute skin wound is an unfavorable wound.

27. The method of claim 1, wherein said acute skin wound comprises subcutaneous tissue.

28. The method of claim 1, wherein said acute skin wound is a head wound.

29. An article of manufacture comprising packaging material and an amount of a botulinum toxin, wherein said packaging material comprises a label that indicates said botulinum toxin is useful for treating a patient having an acute skin wound, and wherein local administration of said amount of said botulinum toxin enhances healing of said skin wound.

30. The article of manufacture of claim 29, wherein said botulinum toxin is botulinum toxin A.

31. The article of manufacture of claim 29, wherein said botulinum toxin is botulinum toxin B.

32. The article of manufacture of claim 29, said article of manufacturing further comprising a local anesthetic.

33. The article of manufacture of claim 29, said article of manufacture further comprising a local vasoconstrictive agent.

34. The article of manufacture of claim 29, said article of manufacture further comprising a local anesthetic and a local vasoconstrictive agent.

* * * * *

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(54) **CYTOTOXIN (NON-NEUROTOXIN) FOR THE TREATMENT OF HUMAN HEADACHE DISORDERS AND INFLAMMATORY DISEASES**

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(57) **ABSTRACT**

Pharmaceutical applications of a chemodenervating agent reduce pain by altering release of pain- and inflammation-mediating autocoids, with a duration of action between 12-24 weeks. The limiting factor in dosing for this application is weakness and paralysis created by higher doses of the chemodenervating pharmaceutical mediated by action of the neurotoxin component of this chemodenervating pharmaceutical. The invention described herein represents a novel mechanism and pharmaceutical formulation which eliminates the neurotoxin component of the chemodenervating pharmaceutical, while retaining the cytotoxin component which provides an essential bioeffect for the relief of pain and inflammation. The invention allows for improvement in administering the pharmaceutical agent for the reduction of pain and/or inflammation without causing muscular weakness and paralysis.

Figure 1a

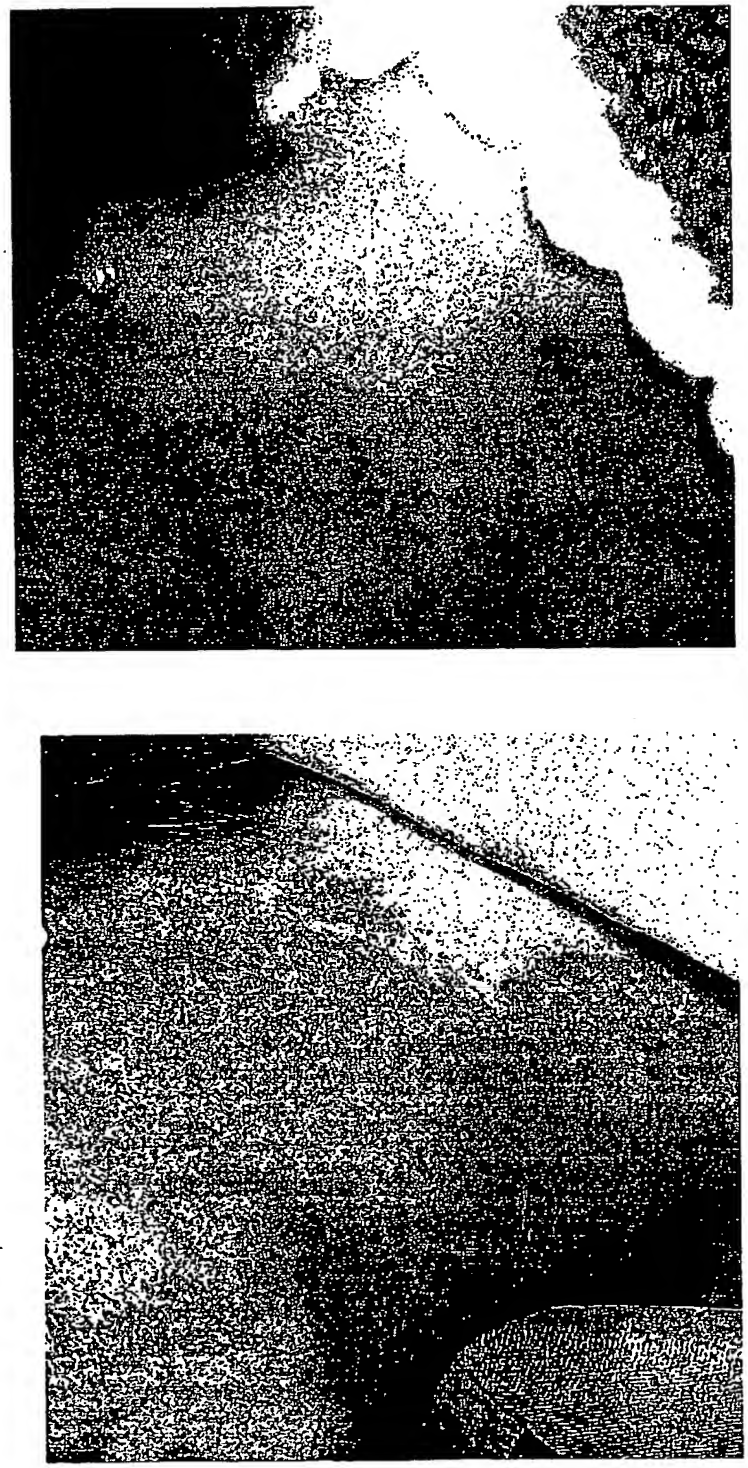


Figure 1b

Figure 1c

Figure 2

Figure 3

Figure 4 , Duration of Effect

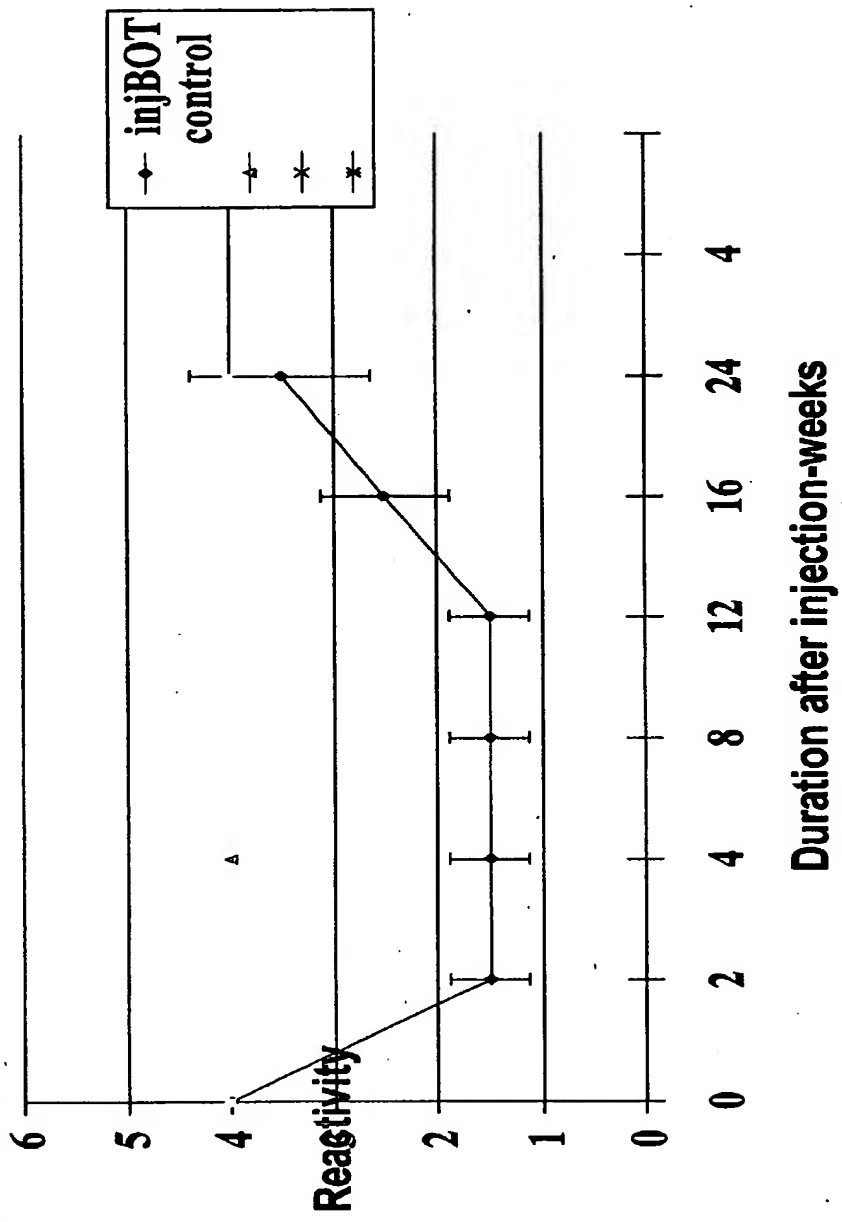
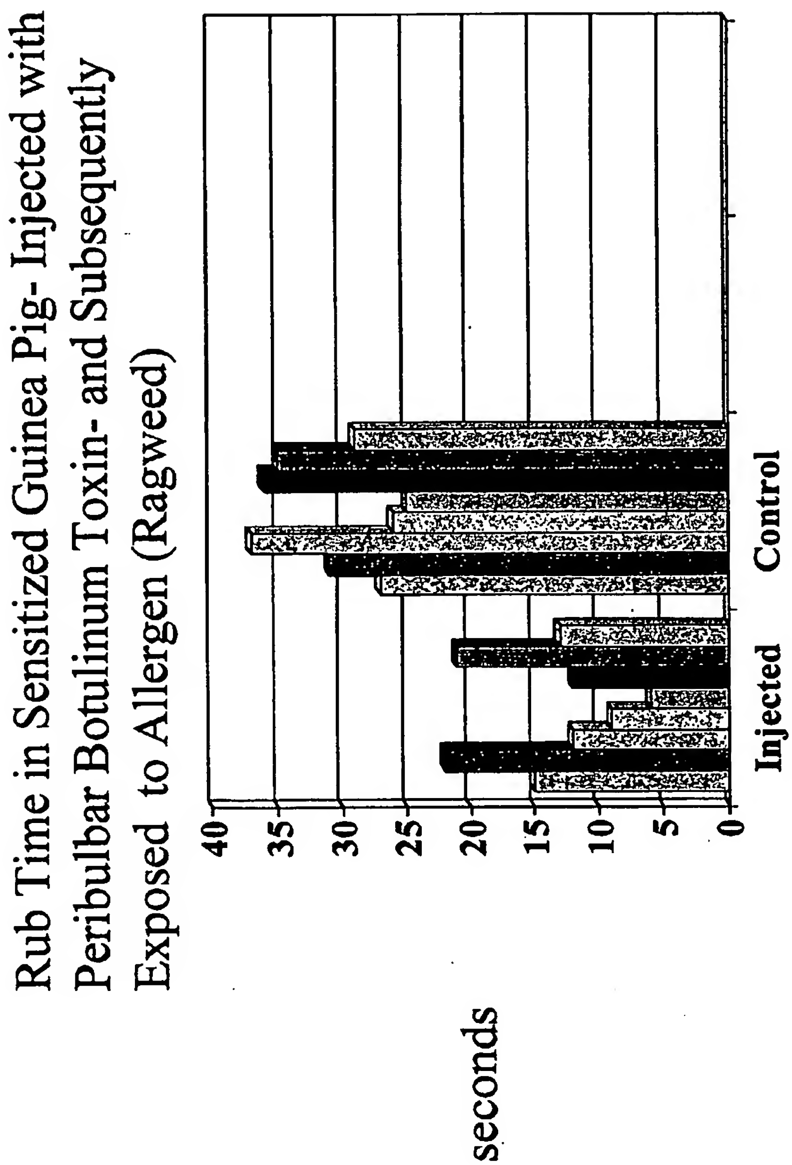


Figure 5 Discomfort Behavior



Each Color represents different animal

Figure 6

CYTOTOXIN (NON-NEUROTOXIN) FOR THE TREATMENT OF HUMAN HEADACHE DISORDERS AND INFLAMMATORY DISEASES

[0001] I claim priority to U.S. patent application Ser. No. 09/458,784, filed Dec. 10, 1999, and hereby incorporate said application by reference herein in its entirety.

FIELD OF INVENTION

[0002] This invention relates to the composition of chemodenervating agents used for the treatment of diseases. The invention offers an improvement on the prior art by eliminating the muscle-weakening side effect of prior-art chemodenervating agents. This is achieved by eliminating the neurotoxin component of the chemodenervating agent.

BACKGROUND OF THE INVENTION

[0003] Migraine and tension headaches are a major cause of loss of productivity for those afflicted, usually due to pain and associated systemic symptoms. The syndrome of migraine and other essential headaches is characterized by severe throbbing headaches often made worse by physical activity and associated with aversion to light and sound. The syndrome often, but not always, includes nausea and/or sometimes vomiting as major components. The pain is often unilateral or localized to a portion of the head. The condition is episodic in nature, with episodes typically lasting 4-72 hours.

[0004] Tension headache is the most common type of essential headache and is characterized by head pain not associated with any structural lesions, often not associated with nausea, and occurring more frequently and less episodically than migraine headache.

[0005] Recently botulinum toxin injections within the head and neck region have been advocated as a method to treat migraine headache, tension headache, and myofascial pain, both acutely and for prophylaxis. Efficacy for both myofascial pain and migraine headaches has been shown in double-blind placebo-controlled trials. However, the mechanism of action has not been clearly explained, as botulinum toxin has been thought to exert its beneficial effects for most indications by causing decreased muscle tone and contractility. The major limiting factor involved in injecting botulinum toxin is the muscle-weakening effect. For instance, ptosis (drooping of the upper eyelid) is the major side effect caused by the injection of neurotoxin for the treatment of human headache disorders. This results from the neuromuscular paralytic effects of the neurotoxin component of the botulinum toxin molecule.

[0006] Recently, the present inventor has shown botulinum toxin to have anti-inflammatory components, both in vivo in man and in animal experiments. This unique property has not been previously appreciated or defined and in fact may explain the mechanism of action by which botulinum toxin exerts its beneficial effects in essential headache disorders (migraine and tension headaches) as well as in other medical conditions. Evidence that botulinum toxin acts on the inflammatory response associated with essential headaches can be categorized into clinical observations and animal experiments as disclosed herein.

BRIEF SUMMARY OF THE INVENTION

[0007] A limiting property of botulinum toxin for the treatment of pain or inflammatory disease relates to its weakening effect on muscle tissue caused by blocking acetylcholine release by its neurotoxin component. The utility of the invention described herein is based upon a compositional change to the botulinum exotoxin proteins used in medical practice that eliminates neurotoxin activity, while maintaining a biological activity that is capable of suppressing pain and inflammation. Such compositional improvement allows the exotoxin protein to be used without the attendant risk of inducing muscle atrophy and weakness, while retaining a biological effect useful for the clinical relief of pain and discomfort.

DETAILED DESCRIPTION

[0008] Botulinum toxin exists as multiple immunotypes (A-G), which have been investigated as to specific medical properties. The immunotypes share little cross reactivity and have been shown to differ in chemical composition and biological behavior when injected at sublethal doses to produce a regional dose-dependent effect. Differences in biological activity between the various immunotypes include (1) differing durations of action at the injection site and (2) differing regional denervation potencies, measured as the relative quantity of LD 50 units required to produce a given regional or clinical effect.

[0009] Botulinum toxin application to human essential headache disorders was originally identified in myofascial tension type headaches (see Acquardro and Borodic, Treatment of myofascial pain with botulinum A toxin. Anesthesiology. March 1994; 80(3):705-6), and later, as a coincident finding, for migraine headache. The coincident finding and application for migraine was noted as botulinum toxin was being used as a neurotoxin to remove forehead wrinkles. Regional facial muscular weakening effaces facial and forehead dynamic lines associated with aging. Such dynamic lines are produced by facial muscle tone as forces are transmitted from facial muscles to dermal attachments of these muscles in facial skin. The neurotoxin component was thought active in the treatment of headaches and pain during this period. Prior art U.S. Pat. No. 5,714,468 teaches that the neurotoxin (that component of botulinum toxin which causes neuromuscular paralysis) is the active agent and mechanism by which botulinum toxin is effective for the treatment of migraine and other forms of pain.

[0010] Of the immunotypes of botulinum toxin, a mutant and unusual derivative subtype of botulinum toxin is botulinum toxin C2, which exhibits no neurotoxin properties. Botulinum toxin C2 possibly represents a mutated gene derivative which demonstrates no neurotoxin capability, yet is toxic by other cytopathic mechanisms (Ohishi, I. Response of mouse intestinal loop to botulinum C2 toxin: enterotoxic activity induced by cooperation of nonlinked protein components. *Infect Immun* May 1983; 40(2):691-5). The proteinaceous materials derived from botulinum C2 strains are, however, biologically active and have been demonstrated to cause lethal effects by mechanisms other than neuromuscular paralysis. The materials are described as cytotoxic in nature. Described herein is the demonstration of botulinum type C2 as a specific inhibitor of inflammation in a sensitized animal model, and reduction to practice utilizing botulinum C2 as a (1) therapy for inflammation and (2) therapy for migraine and tension headache treatment.

[0011] Botulinum toxin has been used for the past 16 years to treat various forms of facial movement disorders, includ-

ing Blepharospasm, hemifacial spasm, bruxism, and synkinetic facial movements after chronic facial palsy. This substance has also proven substantial utility for the treatment of spasmodic torticollis, spasticity associated with cerebral palsy, stroke, occupational hand cramping, and speech disorders (spasmodic dysphonia). In each of these applications, the mechanism of action had been postulated to involve weakening of a fixed volume of muscle around the area injected for a period of 10-18 weeks, with complete reversal of the weakening effect after that time. During the period after the injection, the weakening is correlated to (1) blockage of release of acetylcholine from the presynaptic nerve terminal at the neuromuscular junction, (2) atrophy similar to motor nerve denervation atrophy in the area over which the toxin diffuses, (3) decreased contractility within the muscles over which the toxin diffuses, (4) motor nerve terminal sprouting from the motor axon terminal, (5) spread of acetylcholinesterase and acetylcholine receptors from the post synaptic membrane, and (6) reversibility of the above findings within the denervation field after 10-18 weeks.

[0012] Collectively the above describes a cycle that has been well characterized in the observations of Duchenne (Scott A B Botulinum toxin injection of eye muscles to correct strabismus. Trans Am Ophthalmol Soc 1981;79:734-70). Additionally, it has been well established that botulinum toxin has local effects on autonomic nerve ganglion and nerve function. (MacKenzie I, Burnstock G, Dolly J O The effects of purified botulinum neurotoxin type A on cholinergic, adrenergic and non-adrenergic, atropine-resistant autonomic neuromuscular transmission.Neuroscience April 1982; 7(4):997-1006).

[0013] The medical utility of botulinum toxin has been based primarily on the neuromuscular effects of botulinum neurotoxin, as the neurotoxin generates the cycle described in steps (1)-(6) above. The definition of a neurotoxin is an agent capable of producing death by action on a portion of the central or peripheral nervous system in such a manner as to destroy or critically impair organism function. In the case of botulinum neurotoxin, the action is at the level of the neuromuscular junction, leading to disseminated weakness with paralysis of critical muscles such as the muscles driving respiratory ventilation. The lethal effect, which occurs at a critical point of muscular weakness, is asphyxiation and suffocation. The pharmacological principle governing the utility of botulinum toxin in the treatment of human diseases is that a regional effect occurs at a diluted concentration-dose remote from the lethal concentration-dose. Stated another way, this principle is the property of neurotoxin that allows a regional effect at a neurotoxin dilution and concentration substantially lower than that concentration that would cause a lethal systemic effect for the various types of botulinum neurotoxin used. That lower concentration allows for regional muscular weakening, which has been thought to be the sole mechanism by which the neurotoxin exerts its beneficial action in diseases involving spastic or involuntary movement.

[0014] Despite this scientific understanding of botulinum toxin as a neurotoxin, there remains insufficient understanding of the biological tissue effects to explain observed utility for other medical conditions such as the treatment of human pain such as occurs in essential headache disorders, myofascial pain, and certain pain components associated with dystonias. Also, there exists no explanation of the mechanism by which botulinum toxin is effective in reducing inflammation within the denervation field. The action of botulinum toxin as a neurotoxin, a substance acting at the neuromuscular junction causing muscular weakness, fails to provide a sufficient basis for the mechanism by which utility is achieved for conditions which are not associated with abnormality in movements.

[0015] Described herein is the bioeffect thought critical to the property of botulinum toxin that is directly or indirectly related to its ability to relieve human pain. Also described are methods by which this property can be chemically dissociated from the neurotoxin component (muscle-weakening component) of the botulinum toxin pharmaceutical agent, thereby generating a new perfected botulinum-derived pharmaceutical agent capable of eliminating the undesirable muscle weakness associated with injection of prior-art botulinum toxin preparations into a diseased area.

[0016] Efforts to explain the critical property of botulinum toxin capable of causing an improvement in pain associated with essential headache disorders and migraine headaches initially came from observations of the patient seen in **FIG. 1**. This 53 year old woman experienced flushed face and disseminated itching following physical exertion. The face demonstrated hives, associated with the flushing. Her past medical history was significant for Bell's palsy for which she received a botulinum type A injection for the treatment of forehead asymmetry. It was noted that after the botulinum toxin was injected into the forehead, there would be white blotches appearing on the forehead in which there was no flushing, and no hive formation (blocked urticaria within the denervation field). (See **FIG. 1**). This patient exhibited this effect after physical exertion consistently for a period of three months after the botulinum type A injections and the effect slowly faded thereafter. This duration of effect is typical for botulinum type A injections.

[0017] The syndrome of cholinergic urticaria is typically associated with urticarial eruption after exertion. Sometimes the condition is also associated with symptoms of asthma. The pathophysiology has been linked to increased release of circulating histamine, as well as mast cell degranulation. As the above-noted bioeffect appeared novel and not well explained by existing understanding of botulinum toxin efficacy, efforts were made to confirm the effect on human mast cells in an in vivo laboratory experiment. A Hart Bartley guinea pig (a guinea pig prone to type 1 hypersensitivity reactions) was sensitized to pollen spores (short ragweed pollen—*Ambrosia artemisfolia*), with aerosolized spores sprayed into the conjunctiva of the animals for a period of two weeks. Prior to this exposure, the animals had no reaction to the pollen, with the conjunctival membranes appearing white and quiet after exposure. After two weeks however, animals were again exposed to the short ragweed pollen, which caused acute edema, erythema, itching, flame hemorrhages within the conjunctiva, and distortion of the eyelids. This animal model has been pathologically characterized as being associated with measurable mast cell degranulation histologically, when pollen spores were exposed to sensitized conjunctiva.

[0018] The typical reaction is seen in **FIG. 2**. **FIG. 3** shows the protection by botulinum toxin from the inflammatory response after exposure to the short ragweed pollen. The duration of the protective effect is demonstrated in **FIG.**

4 for a series of 6 animals followed for 6 months. Given the demonstrated efficacy in cholinergic urticaria and demonstrated anti-inflammatory effect in the allergic animal model measuring immediate hypersensitivity reactions, reactions thought to represent mast cell degranulation phenomenon, it appears that botulinum toxin either directly or indirectly is influencing the system which involves mast cells, histamine, possibly serotonin, and other related autocoids in such a fashion to cause a blocked physiological response important to the pathogenesis of certain forms of inflammation and pain. Due to release of autocoids, such as various forms of prostaglandins and leukotrienes as well as other formed and generated local mediators, and as an obvious clinical observation, it is expected that the inflammatory response will be associated with pain degeneration by mechanisms relating to alterations of mast cell secretion or degranulation.

[0019] In a known physiological assay, the relationship between mast cell degranulation and pain is clearly demonstrated. After a type 1 hypersensitivity response is demonstrated on the forearm of a person with known allergy to an introduced allergen, there appears to be a typical wheal and flare response associated with the sensory perception of itching. This is known as the immediate response. After a period of 6-8 hours, a late response is occasionally noted, characterized not by itching but rather tenderness and pain. The immediate response is thought to be associated and effected by preformed mediators such as histamine, whereas the late response is thought to be associated with the leukotrienes and prostaglandins. The prostaglandins and leukotrienes are important in the late phase reaction and are associated with pain generation. Compounds known to block prostaglandin derivatives such as indomethacin and corticosteroids will also block the late phase reactions associated with mast cell degranulation. In cellular systems, dependent on the adhesion of mast cells, there has been observed an increase or decrease in secretion induced by *C. botulinum* C2 toxin. In suspended mast cells, pretreatment with botulinum C2 toxin causes inhibition of secretion. In contrast, in adherent mast cells, the destruction of the cytoskeleton by botulinum C2 toxin causes increase in secretion. Thus, the signaling is largely effected by adhesion of mast cells in cellular in vitro studies, and mast cells have the capability of being influenced by the non-neurotoxin *botulinum* C2.

[0020] There exists a relationship between mast cell activity and migraine and other forms of essential headaches. The pathophysiolgy of essential headaches and migraine has been thought to relate to mast cell function and mast cells degranulation. (Theoharides, T C. The mast cell: a neuroimmunoendocrine master player. Int J Tissue React 1996; 18(1): 1-21; Moskowitz, M A. Neurogenic inflammation in the pathophysiology and treatment of migraine. Neurology June 1993; 43(6 Suppl 3):S16-20; Delepine, L., Aubineau, P. Plasma protein extravasation induced in the rat dura mater by stimulation of the parasympathetic sphenopalatine ganglion. Exp Neurol October 1997; 147(2):389-400.)

[0021] Authors cited above have found that a relationship exists between mast cells and the possible mechanism by which pain is generated in headache disorders, postulating that mast cells play a functional role in the generation of pain nerve adaptation at C-fibers. Although postulated, it appears that no absolute proof relating mast cells to pain generation has been totally established.

[0022] Clinical observations have also linked allergy and mast cell function to the syndrome of migraine headache. The following factors indicate the relationship between mast cells and migraine based on the relationship between type 1 hypersensitivity reactions and migraine. (1) Hayfever allergy season brings out migraines. (2) Stress can be associated with both migraine and urticarial reactions. (3) Patients born to mothers with common migraine are more likely to have offspring with allergic asthma, a mast cell related disease. (4) A known migraine patient receiving a forehead bee sting experienced violent migraine headache within two minutes of the sting. (5) Forms of food allergy are thought to precipitate migraine headaches. (6) Components of headaches (light sensitivity) can also be associated with migraine. (7) Patients with migraine often have elevated blood histamine levels. (8) Mast cells are responsive to cytotoxins.

[0023] In each case, mast-cell-generated inflammation is conceived as a form of inflammation and/or tissue change that provokes genetically predisposed individuals to develop a violent painful sensory experience. Described herein is a cytotoxic botulinum-derived compound capable of blocking inflammation without causing a neurotoxic (neuromuscular) effect.

[0024] There exist both advantages and limitations of *botulinum* neurotoxins in the treatment of human essential headache disorders and human inflammation. Botulinum toxin has many advantages over existing therapy for the treatment of essential headache disorders. These include (1) lack of systemic side effects, particularly compared to the krypton class of drugs, (2) long duration of action (3-5 months), (3) maintenance free therapy (no pills, no autoinjections), (4) high degree of efficacy.

[0025] The major limiting factor is that the prior-art medication produces weakness. In a series of 104 patients treated with type A *botulinum* neurotoxin, the major side effect was ptosis from diffusion of the *botulinum* toxin into the orbital space (Borodic). Diffusion of *botulinum* toxin and attendant weakening effect is not seen only with the treatment of human pain syndromes, but also has been noted with treatment of movement diseases (blepharospasm) causing drooping lids (ptosis), and treatment of cervical dystonia (torticollis) causing difficulty swallowing food (dysphagia).

[0026] Hence for the treatment of movement disease the neurotoxin and weakening bioeffects of *botulinum* toxin are both helpful and a cause of complication. In diseases in which there is no involuntary muscular movements or tone, such as tension or migraine headaches, or forms of human inflammation, the neurotoxin effect would be more detrimental to human clinical applications, causing weakness solely as a complication. Here lies the fundamental utility of the present invention.

[0027] Botulinum toxin exists as immunotypes A-G. Each immunotype is a neurotoxin and causes neuromuscular blockade and weakness when locally injected. However one strain of *botulinum* toxin, perhaps a mutant or derivative strain, has produced a non-neurotoxin protein which demonstrates a selective interaction with the mast cell, does not interact with the neuromuscular junction (neurotoxin), and does not produce weakness (as shown in **FIG. 5**). This *botulinum* protein is biologically active and theorized to act at important tissue sites relative to human pain, migraine

headaches, tension headaches, and headaches involving human inflamnmation—involving mast cells, or mast-cell-contained mediators of inflammation.

[0028] This protein is characterized by those skilled in *botulinum* toxin technology as a cyotototoxin, which causes intestinal inflammation as a cause of toxicity, without inducing muscular weakness. *Clostridium botulinum* C2 toxin, Clostridium perfringens iota toxin, and Clostridium spiroforme toxin act on ADP-ribosylate actin monomers. Toxin-induced ADP-ribosylation disturbs the cellular equilibrium between monomeric and polymeric actin and traps monomeric actin in its unpolymerized form, thereby depolymerizing actin filaments and destroying the intracellular microfilament network (intracellular actin cytoskeleton). Furthermore, the toxins ADP-ribosylate gelsolin actin complexes. These cytoskeletal modifications may contribute to the cytopathic action of this toxins.

[0029] The *botulinum* toxin used to practice the present invention may be prepared as follows. A preparation is made consisting of a *Clostridium botulinum* strain which produces solely C2 cytotoxin. Culture is accomplished with appropriate agents to procure the maximum number of LD 50 units per ml of culture solution. LD 50 units are determined using the mouse bioassay, and the preparation may be freeze-dried for the purpose of preservation and stability. A known quantity of bioactivity as determined by LD 50 is injected into the area in which pain, headache, or inflammation have been diagnosed by the clinician. A quantity for injection is chosen by the clinician based on LD 50 units.

[0030] Botulinum C2 is characterized as a non-neurotoxin, capable of causing increased vascular permeability, fluid accummulation in ligated intestine, and rounding of tissue cultured cells. Bioassay for the activity can be accomplished by a "time to mouse death method" after intravenous infusion, or by intrperitoneal injection. Anti-neurotoxin sera to *botulinum* toxin may be used to confirm complete neutralization of the neurotoxin prior to bioassay. Preparation may be accomplished by biochemical isolation from spent cultures, or by recombinant production using either *E coli* or a Clostridium expression system, given that genes encoding the C2 protein have been elucidated.

[0031] Botulinum C2 cytotoxin has been shown to consist of two protein components not covalently bound, segment I and segment II, both required for the biological activity of the toxin. The following represents examples of preparations that can be used to isolate the C2 property from neurotoxin properties of *botulinum* toxin. Because the biological activity of component II has not been recovered after freeze-drying, a liquid formulation of the material would be preferred.

[0032] Following is a detailed description of an example method for isolating *botulinum* toxin C2.

[0033] 1. Inoculum medium. Prepare 250 mls/carboy of media containing 2% trypticase peptone, 1% proteose peptone, 1% yeast extract, 1.0% glucose and 0.1% cysteine HCl, ph 7.4.

[0034] 2. Incoculation with type C stock. Inoculate each flask with 0.5 ml of thawed working stock culture of *C botulinum* type C. Incubate for 24 hours.

[0035] 3. Production medium. Prepared 9.5 L carboy of media containing 2% trypticase peptone, 1% protease peptone, 1% yeast extract, 1.0% glucose (prepared and autoclaved separately in 400 ml of distilled water) and 0.1% cysteine HCl, pH 7.4.

[0036] 4. Inoculate production medium. Add glucose solution and 250 ml of inoculum to each 9.5 L carboy. Incubate at 30 degrees C. for 6 days.

[0037] 5. Harvest toxin. Toxin was harvested by addition of 2 g/L RNA and dropping the pH to 3.4 by addition of 3N sulfuric acid. Toxin is allowed to settle for at least 16 hours and then collected by centrifugation.

[0038] 6. Wash toxin. Toxin is washed with 1 liter of distilled water and allowed to settle. Washed toxin is collected by centrifugation (10K rpm for 10 min at 10 degrees C.) and the toxin is extracted from the pellets.

[0039] 7. Na phosphate extraction of the toxin. Thoroughly suspend the toxin pellets in 600 ml of 0.2 M Na Phosphate buffer+0.5 M NaCl, pH 7.0, and extract at room temperature for 2 hours. The extract is centrifuged and the new extraction repeated on pelleted material with an additional 400 ml of the same buffer for 2 hours.

[0040] 8. Ammonium sulfate precipitation. Precipitation of the extract by the addition of ammonium sulfate (39 g/100 ml).

[0041] 9. Prepare toxin for chromatography. Collect the precipitate by centrifugation at 8000 rpm for 10 min. Dissolve the precipitate in 50 nM of sodium citrate, pH 5.5, using as small volume as possible.

[0042] 10. Dialysis of toxin solution. Toxin solution is dialyzed against 3X changes in citrate buffer overnight at 4 degree C. and centrifugation ensues.

[0043] 11. DEAE-Sephadex A-50 chromatography. Centrifuged material is chromatographed at room temperature on a −1 DEAE sephadex A-50 column (5 cm×50 cm, Sigma Chemical Co., St Louis, Mo.) equilibrated with the same buffer. One tenth of the column volume or less is chromatographed in a single passage with the toxin complex eluting in the first column without a gradient. Fractions from the protein peak which have a 260/278 absorbance ratio of less than 0.70 are pooled. The material must have a specific toxicity of >10 (6) LD 50/mg. Pooled toxin is precipitated with 60% ammonium sulfate.

[0044] 12. Separation of Component I and II may be accomplished with further chromatographic separation to achieve further purification.

[0045] The Swiss Webster mouse bioassay for lethality of the purified preparation is utilized to quantify bioactivity. Anti-neurotoxin antibody is used for characterization to insure lethal bioeffects in the animal bioassay are explicitly not the result of paralysis induced by neurotoxin. Characterization of the preparation prior to dilution is accomplished with gel electrophoresis, nucleic acid content assay, specific activity assay, and protein content assay for purity and consistency. Dilution accompanied by ultrafiltration establishes sterility. A region is targeted by the physician intending to treat a body region afflicted with the inflammatory process. A known selected bioactivity measured in LD 50 units is injected into the region. After 10 days, the region is inspected for the cardinal signs of inflammation. Booster doses may be given if necessary.

DETAILED DESCRIPTION OF THE DRAWINGS

[0046] **FIG. 1a.** Note the white blotches on the patients forehead indicating blocked urticaria formation within the denervation field. The patient suffers from typical cholinergic urticaria. The blocked area of urticaria, which includes edema, erythema, and altered sensation corresponds to the usual diffusion field of *botulinum* toxin at doses between 5-20 LD 50 units.

[0047] **FIG. 1b.** Note the pattern of erythema on the neck of this patient with dystonia and pain. The picture has been digitally enhanced to emphasize the erythema. Note that in this patient both the erythema and the pain was improved within the denervation field surrounding the injection (arrow).

[0048] **FIG. 1c.** Note the pattern of erythema on the neck of this patient with cervcial dystonia. This patient has been well documented to exhibit circulating neutralizing antibodies to the neuromuscular paralyzing effects of the immunotype A *Clostridium botulinum* toxin. No muscular atrophy is seen in injected muscle yet there is a block in the erythema surrounding the injection site in a dimension equivalent to the diffusion potential for 20-40 LD 50 units of *botulinum* toxin. As neutralizing antibodies to the neuromuscular effect of *botulinum* did not block this anti-pain and anti-inflammatory effect, the anti-inflammatory effect may be the result of a different immuno-reactive epitope on the *botulinum* toxin proteins.

[0049] **FIG. 2.** 15 minutes after short ragweed pollen spores were exposed to the sensitized animal. Note the red eye, edema and distortion of the lower eyelid position.

[0050] **FIG. 3.** Botulinum toxins type A, type C, and type C2, when injected, protect against the inflammatory effects associated with hypersensitivity to short ragweed pollen, hence demonstrating the anti-inflammatory effects of *botulinum* toxin in the animals' orbit tissues.

[0051] **FIG. 4.** The duration of anti-inflammatory effects in a series of six animals followed for 6 months.

[0052] **FIG. 5.** Behavior reaction to irritable stimuli created by ragweed pollen in sensitized Guinea pig conjunctiva. Rubbing the inflamed irritated eye is quantified over a period of 15 minutes after exposure to the pollen.

[0053] **FIG. 6.** The sensitized Guinea pig conjunctiva is injected with *botulinum* C2 preparation. The irritation quantified by rubbing behavior is diminished significantly ($P<0.05$, $n=6$).

I claim:

1. A pharmaceutical composition for treatment of inflammation or pain comprising purified protein,

wherein said purified protein is derived from *Clostridium botulinum,* and

wherein said purified protein substantially lacks neurotoxin properties.

2. The pharmaceutical composition of claim 1,

wherein said purified protein is derived from *Clostridium botulinum* type C.

3. The pharmaceutical composition of claim 1,

wherein said purified protein comprises *botulinum* toxin C2.

4. The pharmaceutical composition of claim 3,

wherein said *botulinum* toxin C2 is purified from a bacterial strain expressing at least one cloned gene encoding a protein component of said *botulinum* toxin C2.

5. A method for treatment of a disorder comprising the steps of,

selecting a region of a patient for treatment, and

administering a therapeutically effective amount of a pharmaceutical composition to said region,

wherein said pharmaceutical composition comprises a purified protein derived from *Clostridium botulinum,* and

wherein said purified protein substantially lacks neurotoxin properties.

6. The method of claim 5, wherein said administration of said pharmaceutical composition to said region produces substantially no muscular weakening.

7. The method of claim 5, wherein said therapeutic effect is mediated by inhibition of secretion of autocoids from mast cells, without antagonizing neuromuscular transmission.

8. The method of claim 5, wherein said purified protein is derived from *Clostridium botulinum* type C.

9. The method of claim 5, wherein said purified protein comprises botulinum toxin C2.

10. The method of claim 9, wherein said *botulinum* toxin C2 is purified from a bacterial strain expressing at least one cloned gene encoding a protein component of said *botulinum* toxin C2.

11. The method of claim 5, wherein said administration is by injection into said region.

12. The method of claim 5, wherein said administration is by topical application to said region.

13. The method of claim 5, wherein said application is of a dose of between approximately 5 and approximately 20 LD 50 units, as measured using the Swiss Webster mouse bioassay.

14. The method of claim 5, wherein said application is of a dose of between approximately 20 and approximately 40 LD 50 units, as measured using the Swiss Webster mouse bioassay.

15. The method of claim 5, wherein the disorder is selected from the group consisting of tension headache, migraine headache, and essential headache.

16. The method of claim 15, wherein said region is the head.

17. The method of claim 15, wherein said region is the neck.

18. The method of claim 5, wherein the disorder is regional muscle pain or inflammation.

19. The method of claim 5, wherein the disorder is selected from the group consisting of inflammation, pain, edema, redness, and itching.

20. The method of claim 5, wherein the disorder is selected from the group consisting of asthma, eczema, hay fever, excessive salivation, tearing, otitis, anal fissures, and cystitis.

21. The method of claim 5, wherein the disorder is selected from the group consisting of gastrointestinal ulceration and gastrointestinal inflammation.

22. The method of claim 5, wherein the disorder is selected from the group consisting of myofascial pain, spasmodic torticollis, blepharospasm, and hemifacial spasm.

23. The method of claim 5, wherein the disorder is rheumatoid arthritis.

24. The method of claim 5, wherein the disorder is vasculitis.

25. The pharmaceutical composition of claim 1, wherein said purified protein is prepared by biochemical separation to remove substantially all neurotoxin properties.

26. The method of claim 5, wherein the disorder is selected from the group consisting of allergic conjunctivitis and allergic blepharoconjunctivitis.

* * * * *

PGPUB-DOCUMENT-NUMBER: 20020187164
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DOCUMENT-IDENTIFIER: US 20020187164 A1

TITLE: Cytotoxin (non-neurotoxin) for the treatment of human headache disorders and inflammatory diseases

PUBLICATION-DATE: December 12, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Borodic, Gary E.	Canton	MA	US

US-CL-CURRENT: 424/247.1

CLAIMS:

I claim:

1. A pharmaceutical composition for treatment of inflammation or pain comprising purified protein, wherein said purified protein is derived from Clostridium botulinum, and wherein said purified protein substantially lacks neurotoxin properties.

2. The pharmaceutical composition of claim 1, wherein said purified protein is derived from Clostridium botulinum type C.

3. The pharmaceutical composition of claim 1, wherein said purified protein comprises botulinum toxin C2.

4. The pharmaceutical composition of claim 3, wherein said botulinum toxin C2 is purified from a bacterial strain expressing at least one cloned gene encoding a protein component of said botulinum toxin C2.

5. A method for treatment of a disorder comprising the steps of, selecting a region of a patient for treatment, and administering a therapeutically effective amount of a pharmaceutical composition to said region, wherein said pharmaceutical composition comprises a purified protein derived from Clostridium botulinum, and wherein said purified protein substantially lacks neurotoxin properties.

6. The method of claim 5, wherein said administration of said pharmaceutical composition to said region produces substantially no muscular weakening.

7. The method of claim 5, wherein said therapeutic effect is mediated by inhibition of secretion of autocoids from mast cells, without antagonizing neuromuscular transmission.

8. The method of claim 5, wherein said purified protein is derived from Clostridium botulinum type C.

9. The method of claim 5, wherein said purified protein comprises botulinum toxin C2.

10. The method of claim 9, wherein said botulinum toxin C2 is purified from a bacterial strain

expressing at least one cloned gene encoding a protein component of said botulinum toxin C2.

11. The method of claim 5, wherein said administration is by injection into said region.

12. The method of claim 5, wherein said administration is by topical application to said region.

13. The method of claim 5, wherein said application is of a dose of between approximately 5 and approximately 20 LD 50 units, as measured using the Swiss Webster mouse bioassay.

14. The method of claim 5, wherein said application is of a dose of between approximately 20 and approximately 40 LD 50 units, as measured using the Swiss Webster mouse bioassay.

15. The method of claim 5, wherein the disorder is selected from the group consisting of tension headache, migraine headache, and essential headache.

16. The method of claim 15, wherein said region is the head.

17. The method of claim 15, wherein said region is the neck.

18. The method of claim 5, wherein the disorder is regional muscle pain or inflammation.

19. The method of claim 5, wherein the disorder is selected from the group consisting of inflammation, pain, edema, redness, and itching.

20. The method of claim 5, wherein the disorder is selected from the group consisting of asthma, eczema, hay fever, excessive salivation, tearing, otitis, anal fissures, and cystitis.

21. The method of claim 5, wherein the disorder is selected from the group consisting of gastrointestinal ulceration and gastrointestinal inflammation.

22. The method of claim 5, wherein the disorder is selected from the group consisting of myofascial pain, spasmodic torticollis, blepharospasm, and hemifacial spasm.

23. The method of claim 5, wherein the disorder is rheumatoid arthritis.

24. The method of claim 5, wherein the disorder is vasculitis.

25. The pharmaceutical composition of claim 1, wherein said purified protein is prepared by biochemical separation to remove substantially all neurotoxin properties.

26. The method of claim 5, wherein the disorder is selected from the group consisting of allergic conjunctivitis and allergic blepharoconjunctivitis.

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L20: Entry 17 of 21 File: PGPB Dec 12, 2002

DOCUMENT-IDENTIFIER: US 20020187164 A1
TITLE: Cytotoxin (non-neurotoxin) for the treatment of human headache disorders and inflammatory diseases

Abstract Paragraph:
Pharmaceutical applications of a chemodenervating agent reduce pain by altering release of pain- and inflammation-mediating autocoids, with a duration of action between 12-24 weeks. The limiting factor in dosing for this application is weakness and paralysis created by higher doses of the chemodenervating pharmaceutical mediated by action of the neurotoxin component of this chemodenervating pharmaceutical. The invention described herein represents a novel mechanism and pharmaceutical formulation which eliminates the neurotoxin component of the chemodenervating pharmaceutical, while retaining the cytotoxin component which provides an essential bioeffect for the relief of pain and inflammation. The invention allows for improvement in administering the pharmaceutical agent for the reduction of pain and/or inflammation without causing muscular weakness and paralysis.

CLAIMS:

21. The method of claim 5, wherein the disorder is selected from the group consisting of gastrointestinal ulceration and gastrointestinal inflammation.

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(12) **United States Patent**
Borodic

(10) **Patent No.: US 6,429,189 B1**
(45) **Date of Patent: Aug. 6, 2002**

(54) **CYTOTOXIN (NON-NEUROTOXIN) FOR THE TREATMENT OF HUMAN HEADACHE DISORDERS AND INFLAMMATORY DISEASES**

(75) Inventor: **Gary E. Borodic**, Canton, MA (US)

(73) Assignee: **Botulinum Toxin Research Associates, Inc.**, Qunicy, MA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

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(22) Filed: **Dec. 10, 1999**

(51) **Int. Cl.**^{7} **A01N 37/18**

(52) **U.S. Cl.** **514/2**; 514/2; 514/14; 514/825; 514/885; 424/810; 424/443; 424/430.1; 424/282.1; 530/350; 530/387.1; 530/389.5; 435/6; 435/842

(58) **Field of Search** 514/14, 2, 825, 514/885; 424/282.1, 810, 443, 130.1; 530/387.1, 350, 389.5

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(57) **ABSTRACT**

Pharmaceutical applications of a chemodenervating agent reduce pain by altering release of pain and inflammation-mediating autocoids, with a duration of action between 12–24 weeks. The limiting factor in dosing for this application is weakness and paralysis created by higher doses of the chemodenervating pharmaceutical. This weakness and paralysis is mediated by action of the neurotoxin component of the chemodenervating pharmaceutical. The invention described herein represents a novel mechanism and pharmaceutical formulation which eliminates the neurotoxin component of the chemodenervating pharmaceutical, while retaining the cytotoxin component which provides an essential bioeffect for the relief of pain and inflammation. The invention allows for improvement in administering the pharmaceutical agent for the reduction of pain and/or inflammation without causing muscular weakness and paralysis.

29 Claims, 2 Drawing Sheets

(2 of 2 Drawing Sheet(s) Filed in Color)

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Figure 1a

Figure 1b

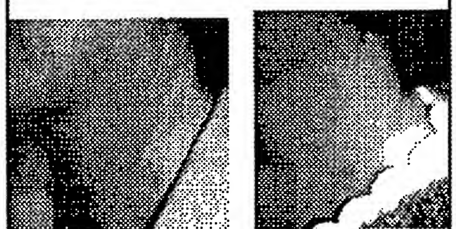

Figure 1c

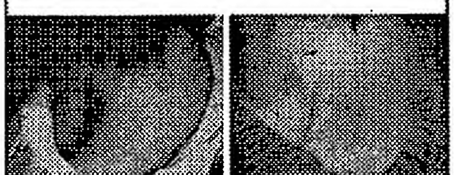

Figure 2

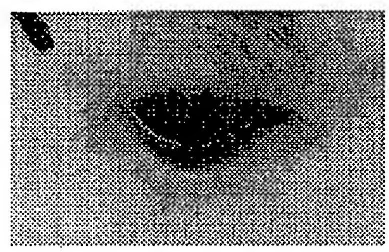

Figure 3

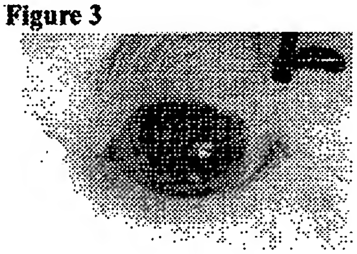

Figure 4 , Duration of Effect

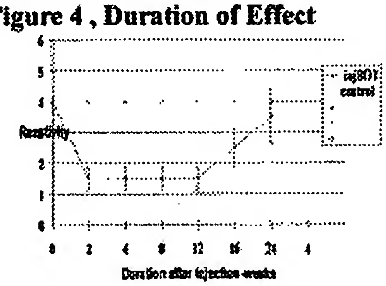

Figure 5 Discomfort Behavior

Rub Time in Sensitized Guinea Pig- Injected with Peribulbar Botulinum Toxin- and Subsequently Exposed to Allergen (Ragweed)

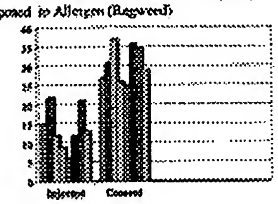

Figure 6

Each Color represents different animal

CYTOTOXIN (NON-NEUROTOXIN) FOR THE TREATMENT OF HUMAN HEADACHE DISORDERS AND INFLAMMATORY DISEASES

FIELD OF INVENTION

This invention relates to the composition of chemodenervating agents used for the treatment of diseases. The invention offers an improvement on the prior art by eliminating the muscle-weakening side effect of prior-art chemodenervating agents. This is achieved by eliminating the neurotoxin component of the chemodenervating agent.

BACKGROUND OF THE INVENTION

Migraine and tension headaches are a major cause of loss of productivity for those afflicted, usually due to pain and associated systemic symptoms. The syndrome of migraine and other essential headaches is characterized by severe throbbing headaches often made worse by physical activity and associated with aversion to light and sound. The syndrome often, but not always, includes nausea and/or sometimes vomiting as major components. The pain is often unilateral or localized to a portion of the head. The condition is episodic in nature, with episodes typically lasting 4–72 hours.

Tension headache is the most common type of essential headache and is characterized by head pain not associated with any structural lesions, often not associated with nausea, and occurring more frequently and less episodically than migraine headache.

Recently *botulinum* toxin injections within the head and neck region have been advocated as a method to treat migraine headache, tension headache, and myofascial pain, both acutely and for prophylaxis. Efficacy for both myofascial pain and migraine headaches has been shown in double-blind placebo-controlled trials. However, the mechanism of action has not been clearly explained, as *botulinum* toxin has been thought to exert its beneficial effects for most indications by causing decreased muscle tone and contractility. The major limiting factor involved in injecting *botulinum* toxin is the muscle-weakening effect. For instance, ptosis (drooping of the upper eyelid) is the major side effect caused by the injection of neurotoxin for the treatment of human headache disorders. This results from the neuromuscular paralytic effects of the neurotoxin component of the *botulinum* toxin molecule.

Recently, the present inventor has shown *botulinum* toxin to have anti-inflammatory components, both in vivo in man and in animal experiments. This unique property has not been previously appreciated or defined and in fact may explain the mechanism of action by which *botulinum* toxin exerts its beneficial effects in essential headache disorders (migraine and tension headaches) as well as in other medical conditions. Evidence that *botulinum* toxin acts on the inflammatory response associated with essential headaches can be categorized into clinical observations and animal experiments as disclosed herein.

BRIEF SUMMARY OF THE INVENTION

A limiting property of *botulinum* toxin for the treatment of pain or inflammatory disease relates to its weakening effect on muscle tissue caused by blocking acetylcholine release by its neurotoxin component. The utility of the invention described herein is based upon a compositional change to the *botulinum* exotoxin proteins used in medical practice that eliminates neurotoxin activity, while maintaining a biological activity that is capable of suppressing pain and inflammation. Such compositional improvement allows the exotoxin protein to be used without the attendant risk of inducing muscle atrophy and weakness, while retaining a biological effect useful for the clinical relief-of pain and discomfort.

DETAILED DESCRIPTION

Botulinum toxin exists as multiple immunotypes (A–G), which have been investigated as to specific medical properties. The immunotypes share little cross reactivity and have been shown to differ in chemical composition and biological behavior when injected at sublethal doses to produce a regional dose-dependent effect. Differences in biological activity between the various immunotypes include (1) differing durations of action at the injection site and (2) differing regional denervation potencies, measured as the relative quantity of LD 50 units required to produce a given regional or clinical effect.

Botulinum toxin application to human essential headache disorders was originally identified in myofascial tension type headaches (see Acquardro and Borodic, Treatment of myofascial pain with *botulinum* A toxin. Anesthesiology. 1994 Mar;80(3):705–6), and later, as a coincident finding, for migraine headache. The coincident finding and application for migraine was noted as *botulinum* toxin was being used as a neurotoxin to remove forehead wrinkles. Regional facial muscular weakening effaces facial and forehead dynamic lines associated with aging. Such dynamic lines are produced by facial muscle tone as forces are transmitted from facial muscles to dermal attachments of these muscles in facial skin. The neurotoxin component was thought active in the treatment of headaches and pain during this period. Prior art U.S. Pat. No. 5,714,468 teaches that the neurotoxin (that component of *botulinum* toxin which causes neuromuscular paralysis) is the active agent and mechanism by which *botulinum* toxin is effective for the treatment of migraine and other forms of pain.

Of the immunotypes of *botulinum* toxin, a mutant and unusual derivative subtype of *botulinum* toxin is *botulinum* toxin C2, which exhibits no neurotoxin properties. *Botulinum* toxin C2 possibly represents a mutated gene derivative which demonstrates no neurotoxin capability, yet is toxic by other cytopathic mechanisms (Ohishi, I. Response of mouse intestinal loop to *botulinum* C2 toxin: enterotoxic activity induced by cooperation of nonlinked protein components. *Infect Immun* 1983 May;40(2):691–5). The proteinaceous materials derived from *botulinum* C2 strains are, however, biologically active and have been demonstrated to cause lethal effects by mechanisms other than neuromuscular paralysis. The materials are described as cytotoxic in nature. Described herein is the demonstration of *botulinum* type C2 as a specific inhibitor of inflammation in a sensitized animal model, and reduction to practice utilizing *botulinum* C2 as a (1) therapy for inflammation and (2) therapy for migraine and tension headache treatment.

Botulinum toxin has been used for the past 16 years to treat various forms of facial movement disorders, including Blepharospasm, hemifacial spasm, bruxism, and synkinetic facial movements after chronic facial palsy. This substance has also proven substantial utility for the treatment of spasmodic torticollis, spasticity associated with cerebral palsy, stroke, occupational hand cramping, and speech disorders (spasmodic dysphonia). In each of these applications, the mechanism of action had been postulated to involve

weakening of a fixed volume of muscle around the area injected for a period of 10–18 weeks, with complete reversal of the weakening effect after that time. During the period after the injection, the weakening is correlated to (1) blockage of release of acetylcholine from the presynaptic nerve terminal at the neuromuscular junction, (2) atrophy similar to motor nerve denervation atrophy in the area over which the toxin diffuses, (3) decreased contractility within the muscles over which the toxin diffuses, (4) motor nerve terminal sprouting from the motor axon terminal, (5) spread of acetylcholinesterase and acetylcholine receptors from the post synaptic membrane, and (6) reversibility of the above findings within the denervation field after 10–18 weeks.

Collectively the above describes a cycle that has been well characterized in the observations of Duchenne (Scott AB *Botulinum* toxin injection of eye muscles to correct strabismus. Trans Am Ophthalmol Soc 1981;79:734–70). Additionally, it has been well established that *botulinum* toxin has local effects on autonomic nerve ganglion and nerve function. (MacKenzie I, Burnstock G, Dolly JO The effects of purified *botulinum* neurotoxin type A on cholinergic, adrenergic and non-adrenergic, atropine-resistant autonomic neuromuscular transmission.Neuroscience 1982 Apr;7(4):997–1006).

The medical utility of *botulinum* toxin has been based primarily on the neuromuscular effects of *botulinum* neurotoxin, as the neurotoxin generates the cycle described in steps (1)–(6) above. The definition of a neurotoxin is an agent capable of producing death by action on a portion of the central or peripheral nervous system in such a manner as to destroy or critically impair organism function. In the case of *botulinum* neurotoxin, the action is at the level of the neuromuscular junction, leading to disseminated weakness with paralysis of critical muscles such as the muscles driving respiratory ventilation. The lethal effect, which occurs at a critical point of muscular weakness, is asphyxiation and suffocation. The pharmacological principle governing the utility of *botulinum* toxin in the treatment of human diseases is that a regional effect occurs at a diluted concentration-dose remote from the lethal concentration-dose. Stated another way, this principle is the property of neurotoxin that allows a regional effect at a neurotoxin dilution and concentration substantially lower than that concentration that would cause a lethal systemic effect for the various types of *botulinum* neurotoxin used. That lower concentration allows for regional muscular weakening, which has been thought to be the sole mechanism by which the neurotoxin exerts its beneficial action in diseases involving spastic or involuntary movement.

Despite this scientific understanding of *botulinum* toxin as a neurotoxin, there remains insufficient understanding of the biological tissue effects to explain observed utility for other medical conditions such as the treatment of human pain such as occurs in essential headache disorders, myofascial pain, and certain pain components associated with dystonias. Also, there exists no explanation of the mechanism by which *botulinum* toxin is effective in reducing inflammation within the denervation field. The action of *botulinum* toxin as a neurotoxin, a substance acting at the neuromuscular junction causing muscular weakness, fails to provide a sufficient basis for the mechanism by which utility is achieved for conditions which are not associated with abnormality in movements.

Described herein is the bioeffect thought critical to the property of *botulinum* toxin that is directly or indirectly related to its ability to relieve human pain. Also described are methods by which this property can be chemically dissociated from the neurotoxin component (muscle-weakening component) of the *botulinum* toxin pharmaceutical agent, thereby generating a new perfected *botulinum*-derived pharmaceutical agent capable of eliminating the undesirable muscle weakness associated with injection of prior-art *botulinum* toxin preparations into a diseased area.

Efforts to explain the critical property of *botulinum* toxin capable of causing an improvement in pain associated with essential headache disorders and migraine headaches initially came from observations of the patient seen in FIG. 1. This 53 year old woman experienced flushed face and disseminated itching following physical exertion. The face demonstrated hives, associated with the flushing. Her past medical history was significant for Bell's palsy for which she received a *botulinum* type A injection for the treatment of forehead asymmetry. It was noted that after the *botulinum* toxin was injected into the forehead, there would be white blotches appearing on the forehead in which there was no flushing, and no hive formation (blocked urticaria within the denervation field). (See FIG. 1). This patient exhibited this effect after physical exertion consistently for a period of three months after the *botulinum* type A injections and the effect slowly faded thereafter. This duration of effect is typical for *botulinum* type A injections.

The syndrome of cholinergic urticaria is typically associated with urticarial eruption after exertion. Sometimes the condition is also associated with symptoms of asthma. The pathophysiology has been linked to increased release of circulating histamine, as well as mast cell degranulation. As the above-noted bioeffect appeared novel and not well explained by existing understanding of *botulinum* toxin efficacy, efforts were made to confirm the effect on human mast cells in an in vivo laboratory experiment. A Hart Bartley guinea pig (a guinea pig prone to type 1 hypersensitivity reactions) was sensitized to pollen spores (short ragweed pollen-Ambrosia artemisfolia), with aerosolized spores sprayed into the conjunctiva of the animals for a period of two weeks. Prior to this exposure, the animals had no reaction to the pollen, with the conjunctival membranes appearing white and quiet after exposure. After two weeks however, animals were again exposed to the short ragweed pollen, which caused acute edema, erythema, itching, flame hemorrhages within the conjunctiva, and distortion of the eyelids. This animal model has been pathologically characterized as being associated with measurable mast cell degranulation histologically, when pollen spores were exposed to sensitized conjunctiva.

The typical reaction is seen in FIG. 2. FIG. 3 shows the protection by *botulinum* toxin from the inflammatory response after exposure to the short ragweed pollen. The duration of the protective effect is demonstrated in FIG. 4 for a series of 6 animals followed for 6 months. Given the demonstrated efficacy in cholinergic urticaria and demonstrated anti-inflammatory effect in the allergic animal model measuring immediate hypersensitivity reactions, reactions thought to represent mast cell degranulation phenomenon, it appears that *botulinum* toxin either directly or indirectly is influencing the system which involves mast cells, histamine, possibly serotonin, and other related autocoids in such a fashion to cause a blocked physiological response important to the pathogenesis of certain forms of inflammation and pain. Due to release of autocoids, such as various forms of prostaglandins and leukotrienes as well as other formed and generated local mediators, and as an obvious clinical observation, it is expected that the inflammatory response will be associated with pain degeneration by mechanisms relating to alterations of mast cell secretion or degranulation.

In a known physiological assay, the relationship between mast cell degranulation and pain is clearly demonstrated. After a type 1 hypersensitivity response is demonstrated on the forearm of a person with known allergy to an introduced allergen, there appears to be a typical wheal and flare response associated with the sensory perception of itching. This is known as the immediate response. After a period of 6–8 hours, a late response is occasionally noted, characterized not by itching but rather tenderness and pain. The immediate response is thought to be associated and effected by preformed mediators such as histamine, whereas the late response is thought to be associated with the leukotrienes and prostaglandins. The prostaglandins and leukotrienes are important in the late phase reaction and are associated with pain generation. Compounds known to block prostaglandin derivatives such as indomethacin and corticosteroids will also block the late phase reactions associated with mast cell degranulation. In cellular systems, dependent on the adhesion of mast cells, there has been observed an increase or decrease in secretion induced by *C. botulinum* C2 toxin. In suspended mast cells, pretreatment with *botulinum* C2 toxin causes inhibition of secretion. In contrast, in adherent mast cells, the destruction of the cytoskeleton by *botulinum* C2 toxin causes increase in secretion. Thus, the signaling is largely effected by adhesion of mast cells in cellular in vitro studies, and mast cells have the capability of being influenced by the non-neurotoxin *botulinum* C2.

There exists a relationship between mast cell activity and migraine and other forms of essential headaches. The pathophysiolgy of essential headaches and migraine has been thought to relate to mast cell function and mast cells degranulation. (Theoharides, TC. The mast cell: a neuroimmunoendocrine master player. Int J Tissue React 1996;1 8(1):1–21; Moskowitz, Ma. Neurogenic inflammation in the pathophysiology and treatment of migraine. Neurology 1993 Jun;43(6 Suppl 3):S 16–20; Delepine, L., Aubineau, P. Plasma protein extravasation induced in the rat dura mater by stimulation of the parasympathetic sphenopalatine ganglion. Exp Neurol 1997 Oct;147(2):389–400.)

Authors cited above have found that a relationship exists between mast cells and the possible mechanism by which pain is generated in headache disorders, postulating that mast cells play a functional role in the generation of pain nerve adaptation at C-fibers. Although postulated, it appears that no absolute proof relating mast cells to pain generation has been totally established.

Clinical observations have also linked allergy and mast cell function to the syndrome of migraine headache. The following factors indicate the relationship between mast cells and migraine based on the relationship between type I hypersensitivity reactions and migraine. (1) Hayfever allergy season brings out migraines. (2) Stress can be associated with both migraine and urticarial reactions. (3) Patients born to mothers with common migraine are more likely to have offspring with allergic asthma, a mast cell related disease. (4) A known migraine patient receiving a forehead bee sting experienced violent migraine headache within two minutes of the sting. (5) Forms of food allergy are thought to precipitate migraine headaches. (6) Components of headaches (light sensitivity) can also be associated with migraine. (7) Patients with migraine often have elevated blood histamine levels. (8) Mast cells are responsive to cytotoxins.

In each case, mast-cell-generated inflammation is conceived as a form of inflammation and/or tissue change that provokes genetically predisposed individuals to develop a violent painful sensory experience. Described herein is a cytotoxic *botulinum*-derived compound capable of blocking inflammation without causing a neurotoxic (neuromuscular) effect.

There exist both advantages and limitations of *botulinum* neurotoxins in the treatment of human essential headache disorders and human inflammation. *Botulinum* toxin has many advantages over existing therapy for the treatment of essential headache disorders. These include (1) lack of systemic side effects, particularly compared to the krypton class of drugs, (2) long duration of action (3–5 months), (3) maintenance free therapy (no pills, no autoinjections), (4) high degree of efficacy.

The major limiting factor is that the prior-art medication produces weakness. In a series of 104 patients treated with type A *botulinum* neurotoxin, the major side effect was ptosis from diffusion of the *botulinum* toxin into the orbital space (Borodic). Diffusion of *botulinum* toxin and attendant weakening effect is not seen only with the treatment of human pain syndromes, but also has been noted with treatment of movement diseases (blepharospasm) causing drooping lids (ptosis), and treatment of cervical dystonia (torticollis) causing difficulty swallowing food (dysphagia).

Hence for the treatment of movement disease the neurotoxin and weakening bioeffects of *botulinum* toxin are both helpful and a cause of complication. In diseases in which there is no involuntary muscular movements or tone, such as tension or migraine headaches, or forms of human inflammation, the neurotoxin effect would be more detrimental to human clinical applications, causing weakness solely as a complication. Here lies the fundamental utility of the present invention. *Botulinum* toxin exists as immunotypes A–G. Each immunotype is a neurotoxin and causes neuromuscular blockade and weakness when locally injected. However one strain of *botulinum* toxin, perhaps a mutant or derivative strain, has produced a non-neurotoxin protein which demonstrates a selective interaction with the mast cell, does not interact with the neuromuscular junction (neurotoxin), and does not produce weakness (as shown in FIG. 5). This *botulinum* protein is biologically active and theorized to act at important tissue sites relative to human pain, migraine headaches, tension headaches, and headaches involving human inflammation-involving mast cells, or mast-cell-contained mediators of inflammation.

This protein is characterized by those skilled in *botulinum* toxin technology as a cyototoxin, which causes intestinal inflammation as a cause of toxicity, without inducing muscular weakness. *Clostridium botulinum* C2 toxin, *Clostridium perfringens* iota toxin, and *Clostridium spiroforme* toxin act on ADP-ribosylate actin monomers. Toxin-induced ADP-ribosylation disturbs the cellular equilibrium between monomeric and polymeric actin and traps monomeric actin in its unpolymerized form, thereby depolymerizing actin filaments and destroying the intracellular microfilament network (intracellular actin cytoskeleton). Furthermore, the toxins ADP-ribosylate gelsolin actin complexes. These cytoskeletal modifications may contribute to the cytopathic action of this toxins.

The *botulinum* toxin used to practice the present invention may be prepared as follows. A preparation is made consisting of a *Clostridium botulinum* strain which produces solely C2 cytotoxin. Culture is accomplished with appropriate agents to procure the maximum number of LD 50 units per ml of culture solution. LD 50 units are determined using the mouse bioassay, and the preparation may be freeze-dried for the purpose of preservation and stability. A known quantity of bioactivity as determined by LD 50 is injected into the

area in which pain, headache, or inflammation have been diagnosed by the clinician. A quantity for injection is chosen by the clinician based on LD 50 units.

Botulinum C2 is characterized as a non-neurotoxin, capable of causing increased vascular permeability, fluid accummulation in ligated intestine, and rounding of tissue cultured cells. Bioassay for the activity can be accomplished by a "time to mouse death method" after intravenous infusion, or by intraperitoneal injection. Anti-neurotoxin sera to *botulinum* toxin may be used to confirm complete neutralization of the neurotoxin prior to bioassay. Preparation may be accomplished by biochemical isolation from spent cultures, or by recombinant production using either *E coli* or a Clostridium expression system, given that genes encoding the C2 protein have been elucidated.

Botulinum C2 cytotoxin has been shown to consist of two protein components not covalently bound, segment I and segment II, both required for the biological activity of the toxin. The following represents examples of preparations that can be used to isolate the C2 property from neurotoxin properties of *botulinum* toxin. Because the biological activity of component II has not been recovered after freeze-drying, a liquid formulation of the material would be preferred.

Following is a detailed description of an example method for isolating *botulinum* toxin C2.

1. Inoculum medium. Prepare 250 mls/carboy of media containing 2% trypticase peptone, 1% protease peptone, 1% yeast extract, 1.0% glucose and 0.1% cysteine HCl, ph 7.4.
2. Incoculation with type C stock. Inoculate each flask with 0.5 ml of thawed working stock culture of *C botulinum* type C. Incubate for 24 hours.
3. Production medium. Prepared 9.5 L carboy of media containing 2% trypticase peptone, 1% protease peptone, 1% yeast extract, 1.0% glucose (prepared and autoclaved separately in 400 ml of distilled water) and 0.1% cysteine HCl , pH 7.4.
4. Inoculate production medium. Add glucose solution and 250 ml of inoculum to each 9.5 L carboy. Incubate at 30 degrees C for 6 days.
5. Harvest toxin. Toxin was harvested by addition of 2 g/L RNA and dropping the pH to 3.4 by addition of 3 N sulfuric acid. Toxin is allowed to settle for at least 16 hours and then collected by centrifugation.
6. Wash toxin. Toxin is washed with 1 liter of distilled water and allowed to settle. Washed toxin is collected by centrifugation (10K rpm for 10 min at 10 degrees C) and the toxin is extracted from the pellets.
7. Na phosphate extraction of the toxin. Thoroughly suspend the toxin pellets in 600 ml of 0.2 M Na Phosphate buffer+0.5 M NaCl, pH 7.0, and extract at room temperature for 2 hours. The extract is centrifuged and the new extraction repeated on pelleted material with an additional 400 ml of the same buffer for 2 hours.
8. Ammonium sulfate precipitation. Precipitation of the extract by the addition of ammonium sulfate (39 g/100 ml).
9. Prepare toxin for chromatography. Collect the precipitate by centrifugation at 8000 rpm for 10 min. Dissolve the precipitate in 50 nM of sodium citrate, pH 5.5, using as small volume as possible.
10. Dialysis of toxin solution. Toxin solution is dialyzed against 3 X changes in citrate buffer overnight at 4 degree C. and centrifugation ensues.
11. DEAE-Sephadex A-50 chromatography. Centrifuged material is chromatographed at room temperature on a -1 DEAE sephadex A-50 column (5 cm×50 cm, Sigma Chemical Co., St Louis , Mo.) equilibrated with the same buffer. One tenth of the column volume or less is chromatographed in a single passage with the toxin complex eluting in the first column without a gradient. Fractions from the protein peak which have a 260/278 absorbance ratio of less than 0.70 are pooled. The material must have a specific toxicity of>10 (6) LD 50/mg. Pooled toxin is precipitated with 60% ammonium sulfate.
12. Separation of Component I and II may be accomplished with further chromatographic separation to achieve further purification.

The Swiss Webster mouse bioassay for lethality of the purified preparation is utilized to quantify bioactivity. Anti-neurotoxin antibody is used for characterization to insure lethal bioeffects in the animal bioassay are explicitly not the result of paralysis induced by neurotoxin.

Characterization of the preparation prior to dilution is accomplished with gel electrophoresis, nucleic acid content assay, specific activity assay, and protein content assay for purity and consistency. Dilution accompanied by ultrafiltration establishes sterility. A region is targeted by the physician intending to treat a body region afflicted with the inflammatory process. A known selected bioactivity measured in LD 50 units is injected into the region. After 10 days, the region is inspected for the cardinal signs of inflammation. Booster doses may be given if necessary.

DETAILED DESCRIPTION OF THE DRAWINGS

The file of this patent contains at least one drawing executed in color. Copies of this patent with color drawing(s) will be provided by the Patent and Trademark Office upon request and payment of the necessary fee.

FIG. 1*a*. Note the white blotches on the patients forehead indicating blocked urticaria formation within the denervation field. The patient suffers from typical cholinergic urticaria. The blocked area of urticaria, which includes edema, erythema, and altered sensation corresponds to the usual diffusion field of *botulinum* toxin at doses between 5–20 LD 50 units.

FIG. 1*b*. Note the pattern of erythema on the neck of this patient with dystonia and pain. The picture has been digitally enhanced to emphasize the erythema. Note that in this patient both the erythema and the pain was improved within the denervation field surrounding the injection (arrow).

FIG. 1*c*. Note the pattern of erythema on the neck of this patient with cervcial dystonia. This patient has been well documented to exhibit circulating neutralizing antibodies to the neuromuscular paralyzing effects of the immunotype A *Clostridium botulinum* toxin. No muscular atrophy is seen in injected muscle yet there is a block in the erythema surrounding the injection site in a dimension equivalent to the diffusion potential for 20–40 LD 50 units of *botulinum* toxin. As neutralizing antibodies to the neuromuscular effect of *botulinum* did not block this anti-pain and anti-inflammatory effect, the anti-inflammatory effect may be the result of a different immuno-reactive epitope on the *botulinum* toxin proteins.

FIG. 2. 15 minutes after short ragweed pollen spores were exposed to the sensitized animal. Note the red eye, edema and distortion of the lower eyelid position.

FIG. 3. *Botulinum* toxins type A, type C, and type C2, when injected, protect against the inflammatory effects associated with hypersensitivity to short ragweed pollen, hence demonstrating the anti-inflammatory effects of *botulinum* toxin in the animals' orbit tissues.

FIG. 4. The duration of anti-inflammatory effects in a series of six animals followed for 6 months.

FIG. 5. Behavior reaction to irritable stimuli created by ragweed pollen in sensitized Guinea pig conjunctiva. Rubbing the inflamed irritated eye is quantified over a period of 15 minutes after exposure to the pollen.

FIG. 6. The sensitized Guinea pig conjunctiva is injected with *botulinum* C2 preparation. The irritation quantified by rubbing behavior is diminished significantly ($P<0.05$, $n=6$).

What is claimed is:

1. A pharmaceutical composition for treatment of inflammation or pain comprising purified protein, wherein said purified protein is derived from *Clostridium botulinum*, and wherein said purified protein exhibits no neurotoxin properties.

2. The pharmaceutical composition of claim 1, wherein said purified protein is derived from *Clostridium botulinum* type C.

3. The pharmaceutical composition of claim 1, wherein said purified protein comprises *botulinum* toxin C2.

4. The pharmaceutical composition of claim 3, wherein said *botulinum* toxin C2 is purified from a bacterial strain expressing at least one cloned gene encoding a protein component of said *botulinum* toxin C2.

5. A method for treatment of inflammation or pain comprising,

administering locally a therapeutically effective amount of a pharmaceutical composition,

wherein said pharmaceutical composition comprises a purified protein derived from *Clostridium botulinum*,

wherein said purified protein exhibits no neurotoxin properties, and

wherein said administration is for a time and under conditions sufficient to reduce the inflammation or pain.

6. The method of claim 5, wherein said administration of said pharmaceutical composition produces no muscular weakening.

7. The method of claim 5, wherein said therapeutic effect is mediated by inhibition of secretion of autocoids from mast cells, without antagonizing neuromuscular transmission.

8. The method of claim 5, wherein said purified protein is derived from *Clostridium botulinum* type C.

9. The method of claim 5, wherein said purified protein comprises *botulinum* toxin C2.

10. The method of claim 9, wherein said *botulinum* toxin C2 is purified from a bacterial strain expressing at least one cloned gene encoding a protein component of said *botulinum* toxin C2.

11. The method of claim 5, wherein said administration is by injection.

12. The method of claim 5, wherein said administration is by topical application.

13. The method of claim 5, wherein said administration is of a dose of from approximately 5 to approximately 20 LD 50 units, as measured using the Swiss Webster mouse bioassay.

14. The method of claim 5, wherein said administration is of a dose of from approximately 20 to approximately 40 LD 50 units, as measured using the Swiss Webster mouse bioassay.

15. The method of claim 5, wherein the inflammation or pain is caused by a disorder selected from the group consisting of tension headache, migraine headache, post-operative head and neck pain, trigeminal neuralgia, temporal mandibular joint syndrome, and essential headache.

16. The method of claim 15, wherein said local administration is to the head.

17. The method of claim 15, wherein said local administration is to the neck.

18. The method of claim 5, wherein the inflammation or pain is regional muscle pain or inflammation.

19. The method of claim 5, wherein the inflammation or pain comprises symptoms selected from the group consisting of inflammation, pain, edema, redness, and itching.

20. The method of claim 5, wherein the inflammation or pain is caused by a disorder selected from the group consisting of asthma, eczema, hay fever, excessive salivation, tearing, otitis, anal fissures, and cystitis.

21. The method of claim 5, wherein the inflammation or pain is caused by a disorder selected from the group consisting of gastrointestinal ulceration and gastric, small, and large intestinal inflammation.

22. The method of claim 5, wherein the inflammation or pain is caused by a disorder selected from the group consisting of myofascial pain, regional dystonia, spasmodic torticollis, blepharospasm, and hemifacial spasm.

23. The method of claim 5, wherein the inflammation or pain is caused by rheumatoid arthritis.

24. The method of claim 5, wherein the inflammation or pain is caused by vasculitis.

25. The method of claim 1, wherein said purified protein is prepared by biochemical separation to remove all neurotoxin properties.

26. The method of claim 5, wherein the inflammation or pain is caused by a disorder selected from the group consisting of allergic conjunctivitis and allergic blepharoconjunctivitis.

27. The pharmaceutical composition of claim 1, wherein said composition exhibits no muscle weakening properties.

28. The pharmaceutical composition of claim 1, wherein said composition is effective in treating conjunctivitis.

29. The pharmaceutical composition of claim 1, wherein said composition is effective in treating allergic conjunctivitis.

* * * * *

L20: Entry 21 of 21 File: USPT Aug 6, 2002

DOCUMENT-IDENTIFIER: US 6429189 B1
TITLE: Cytotoxin (non-neurotoxin) for the treatment of human headache disorders and inflammatory diseases

Abstract Text (1):
Pharmaceutical applications of a chemodenervating agent reduce pain by altering release of pain and inflammation-mediating autocoids, with a duration of action between 12-24 weeks. The limiting factor in dosing for this application is weakness and paralysis created by higher doses of the chemodenervating pharmaceutical. This weakness and paralysis is mediated by action of the neurotoxin component of the chemodenervating pharmaceutical. The invention described herein represents a novel mechanism and pharmaceutical formulation which eliminates the neurotoxin component of the chemodenervating pharmaceutical, while retaining the cytotoxin component which provides an essential bioeffect for the relief of pain and inflammation. The invention allows for improvement in administering the pharmaceutical agent for the reduction of pain and/or inflammation without causing muscular weakness and paralysis.

CLAIMS:

21. The method of claim 5, wherein the inflammation or pain is caused by a disorder selected from the group consisting of gastrointestinal ulceration and gastric, small, and large intestinal inflammation.

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L20: Entry 21 of 21 File: USPT Aug 6, 2002

US-PAT-NO: 6429189
DOCUMENT-IDENTIFIER: US 6429189 B1

TITLE: Cytotoxin (non-neurotoxin) for the treatment of human headache disorders and inflammatory diseases

DATE-ISSUED: August 6, 2002

INVENTOR-INFORMATION:

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US-CL-CURRENT: 514/2; 424/130.1, 424/282.1, 424/443, 424/810, 435/6, 435/842, 514/14, 514/825, 514/885, 530/350, 530/387.1, 530/389.5

CLAIMS:

What is claimed is:

1. A pharmaceutical composition for treatment of inflammation or pain comprising purified protein, wherein said purified protein is derived from Clostridium botulinum, and wherein said purified protein exhibits no neurotoxin properties.

2. The pharmaceutical composition of claim 1, wherein said purified protein is derived from Clostridium botulinum type C.

3. The pharmaceutical composition of claim 1, wherein said purified protein comprises botulinum toxin C2.

4. The pharmaceutical composition of claim 3, wherein said botulinum toxin C2 is purified from a bacterial strain expressing at least one cloned gene encoding a protein component of said botulinum toxin C2.

5. A method for treatment of inflammation or pain comprising, administering locally a therapeutically effective amount of a pharmaceutical composition, wherein said pharmaceutical composition comprises a purified protein derived from Clostridium botulinum, wherein said purified protein exhibits no neurotoxin properties, and wherein said administration is for a time and under conditions sufficient to reduce the inflammation or pain.

6. The method of claim 5, wherein said administration of said pharmaceutical composition produces no muscular weakening.

7. The method of claim 5, wherein said therapeutic effect is mediated by inhibition of secretion of autocoids from mast cells, without antagonizing neuromuscular transmission.

8. The method of claim 5, wherein said purified protein is derived from Clostridium botulinum type C.

9. The method of claim 5, wherein said purified protein comprises botulinum toxin C2.

10. The method of claim 9, wherein said botulinum toxin C2 is purified from a bacterial strain expressing at least one cloned gene encoding a protein component of said botulinum toxin C2.

11. The method of claim 5, wherein said administration is by injection.

12. The method of claim 5, wherein said administration is by topical application.

13. The method of claim 5, wherein said administration is of a dose of from approximately 5 to approximately 20 LD 50 units, as measured using the Swiss Webster mouse bioassay.

14. The method of claim 5, wherein said administration is of a dose of from approximately 20 to approximately 40 LD 50 units, as measured using the Swiss Webster mouse bioassay.

15. The method of claim 5, wherein the inflammation or pain is caused by a disorder selected from the group consisting of tension headache, migraine headache, post-operative head and neck pain, trigeminal neuralgia, temporal mandibular joint syndrome, and essential headache.

16. The method of claim 15, wherein said local administration is to the head.

17. The method of claim 15, wherein said local administration is to the neck.

18. The method of claim 5, wherein the inflammation or pain is regional muscle pain or inflammation.

19. The method of claim 5, wherein the inflammation or pain comprises symptoms selected from the group consisting of inflammation, pain, edema, redness, and itching.

20. The method of claim 5, wherein the inflammation or pain is caused by a disorder selected from the group consisting of asthma, eczema, hay fever, excessive salivation, tearing, otitis, anal fissures, and cystitis.

21. The method of claim 5, wherein the inflammation or pain is caused by a disorder selected from the group consisting of gastrointestinal ulceration and gastric, small, and large intestinal inflammation.

22. The method of claim 5, wherein the inflammation or pain is caused by a disorder selected from the group consisting of myofascial pain, regional dystonia, spasmodic torticollis, blepharospasm, and hemifacial spasm.

23. The method of claim 5, wherein the inflammation or pain is caused by rheumatoid arthritis.

24. The method of claim 5, wherein the inflammation or pain is caused by vasculitis.

25. The method of claim 1, wherein said purified protein is prepared by biochemical separation to remove all neurotoxin properties.

26. The method of claim 5, wherein the inflammation or pain is caused by a disorder selected from the group consisting of allergic conjunctivitis and allergic blepharoconjunctivitis.

27. The pharmaceutical composition of claim 1, wherein said composition exhibits no muscle weakening properties.

28. The pharmaceutical composition of claim 1, wherein said composition is effective in treating conjunctivitis.

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